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13. ABSTRACT ***(Maximum 200 Words)***

Imaging procedures play an important role in the current management of patients with prostate cancer. Despite advances in many of these methodologies, improvements are still needed, especially in the area of Nuclear Medicine. The radiodinated phospholipid ether analogs investigated under this grant represent a new class of radiopharmaceutical, which has provided excellent images of prostate tumors in animal models. Two of the newer analogs, namely NM-404 and NM-412, were found to be significantly superior to the prototype agent, NM-324 in both the rat and mouse tumor models, while avoiding the high first pass clearance by the liver displayed by NM-324. NM-404 has been approved by the Food and Drug Administration as an Investigational New Drug (IND 62,703).

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prostate cancer, diagnostic imaging, pharmacokinetics, radioiodinated agents, phospholipid ether analogs

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Table of Contents

5. Introduction:

Nuclear Medicine imaging procedures play a major role in the management of patients with prostate cancer. Despite advances in these procedures, improvements are still needed. To fulfill this need, a procedure has to be discovered that will selectively target a radionuclide such as radioiodine to prostate tumors. Working with a number of radioiodinated phospholipid analogs synthesized in our laboratory, we demonstrated the remarkable capacity of several of these agents to be taken up and retained by a variety of tumors. One of these radiotracers, NM-324, displayed excellent imaging characteristics in the Walker 256 tumor model and nude mouse/human tumor xenografts models. Following the necessary toxicologic studies, NM-324 was approved for an initial trial in cancer patents. This study revealed that NM-324 was capable of imaging tumors in patients, but the high first pass by the liver severely compromised its clinical utility as a diagnostic radiopharmaceutical. Therefore, the design of second-generation candidates focused on those agents that would have a longer plasma half-life and thereby avoid excessive accumulation in the liver. These follow up studies employed Copenhagen rats bearing the Dunning R3327 prostate tumors and SCID mice with human PC-3 xenografts to evaluate the newer agents. These preliminary studies showed two agents, namely NM-404 and NM-412, to be significantly superior to NM-324 in both the rat and mouse tumor models. It was, therefore, the propose of the present study to prepare more of the target compounds and perform the necessary preclinical animal biodistribution, gamma camera imaging, and toxicology to warrant approval as an Investigational New Drug (I.N.D.) by the Food and Drug Administration. The ultimate goal would be a preliminary pharmacokinetic evaluation of the best agent in patients suffering from prostate cancer.

6. **Body:** To achieve the goals of this study it was necessary to synthesize sufficient amounts of pure NM-404 and NM-412. This was followed up by reviewing our previous method for radioiodination and experimenting with several modifications in order to improve the resulting specific activity of the final product. Once labeled with radioiodine, both NM-404 and NM-412were appropriately formulated and subjected to biodistribution analysis in prostate tumor bearing animals in order to demonstrate their ability to concentrate in the tumors at a level sufficient for external imaging by a gamma camera. It was also necessary to obtain tissue biodistribution data in normal animals in order to calculate the radiation dose to each of the tissues. Finally, acute toxicity of each agent was conducted by the Toxicology Center of the University of New York at Buffalo. All of this preclinical data is required by the Food and Drug Administration in order to receive approval as an Investigational New Drug (I.N.D).

SYNTHESIS OF NM-404 AND NM-412: The details for the synthesis of NM-404 and NM-412 is provided in Appendix 1 and illustrated in the accompanying scheme. NM-404 was chemically less complex than NM-412 and was accomplished first. The synthesis of NM-412 was accomplished by the second year of the project. The final products were purified by column chromatography and the structures verified by proton NMR and elemental analysis.

RADIOIODINATION OF NM-404 AND NM-412: These molecules were readily labeled with radioiodine by utilizing a procedure known as isotope exchange. We have employed this technique for over three decades and have primarily used pivalic acid as the exchange media. During the course of this study, however, a significant improvement in the specific activity of the final product was achieved by conducting the exchange in ammonium sulfate.

FORMULATION OF NM-404 AND NM-412: Following purification of each of the radiopharmaceuticals by High Pressure Liquid Chromatography, they were dissolved in ethanol and Tween 20 (0.1 *ul*/mg of compound). The ethanol was removed under vacuum and the residue dissolved in sterile water to give a final solution containing no more than 2-3% Tween 20. Sterilization was then achieved by filtration through a sterile 0.2 *u*m filter unit. All solutions of the test compounds were prepared in this way for the animal experiments and toxicological studies.

BIODISTRIBUTION OF NM-404 AND NM-412 IN SCID MICE BEARING PC-3 PROSTATE TUMORS: Adult male SCID mice were injected subcutaneously with $1x10^6$ PC-3 tumor cells. Tumors took 6-8 weeks to develop. Approximately 3-5 *u*Ci of the radiopharmaceutical was administered intravenously to each animal while they were under deep Metofane anesthesia. At specified times, tissues were isolated, weighed and counted in order to calculate the concentration of radioactivity in each tissue and to calculate the target to non-target ratios. The results for NM-404 are shown in Table 1. The amount of radioactivity appearing in the thyroid is a result of a minimal amount of *in vivo* deiodination of the radiopharmaceutical. This is readily blocked when animals are pretreated with Lugol's (KI) solution. The levels of radioactivity localizing in the prostate tumor following administration of NM-404 were extremely high whereas only a small amount appeared in the normal prostate. At three days, the tumor/kidney and tumor/liver ratios were greater than 6 and at day eight they were greater than 10.

The results of a similar study with radioiodinated NM-412 are outlined in Table 2. While tumor uptake and retention of radioactivity following administration of NM-412 was significant, at no time did levels reach that seen for NM-404. Moreover, the target/non-target ratios for NM-404 were superior to those for NM-412. On this basis, NM-404 was selected as the preferred candidate for preclinical workup and clinical follow up.

GAMMA CAMERA IMAGING OF TUMORED ANIMALS: Adult male SCID mice with PC3 prostate tumors were injected with approximately 30 *u*Ci of either NM-404 or NM-412. The mice were imaged by gamma camera scintigraphy for a number days following administration of the test agent. Figure 1 shows the dramatic ability of NM-404 to image the tumor at 1 day. By day 4 the tumor is intensely visible whereas radioactivity in the other tissues has dissipated. The inability to visualize the thyroid in these studies emphasized the stability of NM-404 to *in vivo* deiodination. Figures 2a and 2b show the results with NM-412. In contrast with NM-404, the tumor was not readily visualized at day 1, but became readily apparent by day 3 and day 5. The imaging results were consistent with the tissue distribution data.

EXTRACTION OF TUMORS AND PLASMA: In order to characterize the radioactive component in the tumors and plasma, these tissues were extracted with butanol and an aliquot analyzed by thin layer chromatography. Extraction of radioactivity was essentially 100% under these conditions. Figure 3 demonstrates that the radioactivity present in the tumor was unchanged NM-404. No other radioactive metabolite could be identified in the tumor extracts. Similarly, Figure 4 shows the results of thin layer analysis of the plasma extract. At days 1 and 3, radioactivity in the plasma is present largely as unchanged NM-404, whereas by day 5 and day 8 possible metabolites appear to be present. No attempt was made to characterize these metabolites.

Biodistribution of ^{125}I-NM-404 in male SCID mice bearing PC-3 prostate cancer xenografts, expressed as % Dose/gm ± SEM and Target/Non-target Ratio, (n=4).

	1 Day		3 Day		5 Day		8 Day	
	% Dose/gm	Target/Non-target	% Dose/gm	Target/Non-target	% Dose/gm	Target/Non-target	% Dose/gm	Target/Non-target
Adrenal	7.825±0.776	1.17	4.651±0.403	2.83	4.759±0.513	3.80	3.000±0.137	4.99
Blood	5.738±0.204	1.59	3.101±0.133	4.24	3.077±0.094	5.87	2.166±0.066	6.91
Duodenum	5.115±0.669	1.79	2.872±0.102	4.58	2.704±0.134	6.68	1.668±0.054	8.97
Fat	2.393±0.327	3.82	1.255±0.207	10.47	1.109±0.095	16.28	0.868±0.108	17.23
Heart	2.805±0.102	3.26	1.453±0.042	9.05	1.383±0.056	13.06	0.973±0.030	15.37
Kidney	4.217±0.141	2.17	2.145±0.113	6.13	2.281±0.089	7.92	1.458±0.041	10.26
Liver	3.690±0.215	2.48	1.930±0.103	6.81	1.632±0.061	11.07	1.018±0.060	14.69
Lung	5.363±0.326	1.71	2.596±0.200	5.06	2.267±0.089	7.97	1.543±0.064	9.70
Lymph Node	27.962±6.224	0.33	12.489	1.05				
Muscle	0.795±0.026	11.50	0.572±0.042	22.98	0.489±0.025	36.95	0.401±0.029	37.33
Plasma	9.927±0.529	0.92	5.271±0.158	2.49	5.405±0.136	3.34	3.975±0.152	3.76
Prostate	2.605±0.148	3.51	1.399±0.270	9.40	1.963±0.254	9.20	1.406±0.061	10.64
Spleen	4.989±0.365	1.83	2.237±0.115	5.88	1.987±0.102	9.09	1.355±0.066	11.04
Thyroid	42.684±10.718	0.21	54.529±12.537	0.24	26.475±3.987	0.68	33.900±11.015	0.44
Tumor	9.144±0.686	1.00	13.142±0.401	1.00	18.062±0.807	1.00	14.956±0.633	1.00

TABLE 1

TABLE 2

BIODISTRIBUTION OF ^{125}I-NM-412 IN MALE SCID MICE BEARING PC-3 XENOGRAFTS EXPRESSED AS % ADMINISTERED DOSE/GM (MEAN, N=4).

	DAY 1	DAY 3	DAY 5	DAY 8	DAY 14
ADRENAL	6.369	3.028	1.450	1.113	0.700
BLOOD	1.842	0.707	0.371	0.347	0.050
DUODENUM	2.999	1.369	0.615	0.392	0.138
FAT	0.997	1.240	0.422	0.398	0.451
HEART	1.783	0.667	0.326	0.238	0.109
KIDNEY	3.603	1.539	0.591	0.380	0.088
LIVER	4.285	1.438	0.787	0.866	0.279
LUNG	6.032	2.812	1.178	0.674	0.197
MUSCLE	0.527	0.307	0.156	0.114	0.060
PLASMA	8.507	1.253	0.368	0.350	0.070
PROSTATE	1.695	1.212	0.633	0.320	0.130
SPLEEN	4.269	1.686	0.587	0.698	0.175
THYROID	1.933	1.219	0.435	0.300	0.221
TUMOR	2.163	3.230	2.848	2.640	1.839

TUMOR TO NON-TARGET RATIOS OF NM-412 IN MALE SCID MICE BEARING PC-3 XENOGRAFTS.

NON-TARGET TISSUE	DAY 1	DAY 3	DAY 5	DAY 8	DAY 14
ADRENAL	0.34	1.07	1.96	2.37	2.63
BLOOD	1.20	4.57	7.68	7.61	36.84
DUODENUM	0.72	2.36	4.63	6.73	13.32
FAT	2.17	2.60	6.75	6.63	4.08
HEART	1.12	4.84	8.87	11.09	16.91
KIDNEY	0.60	2.10	4.82	6.94	20.96
LIVER	0.50	2.25	3.62	3.05	6.59
LUNG	0.36	2.16	2.42	3.91	9.34
MUSCLE	4.11	10.52	18.27	23.12	30.64
PLASMA	0.24	2.58	7.74	7.55	26.12
PROSTATE	1.28	2.67	4.50	8.26	14.16
SPLEEN	0.51	1.92	4.85	3.78	10.49
THYROID	1.12	2.65	6.55	8.81	8.32

FIGURE 1

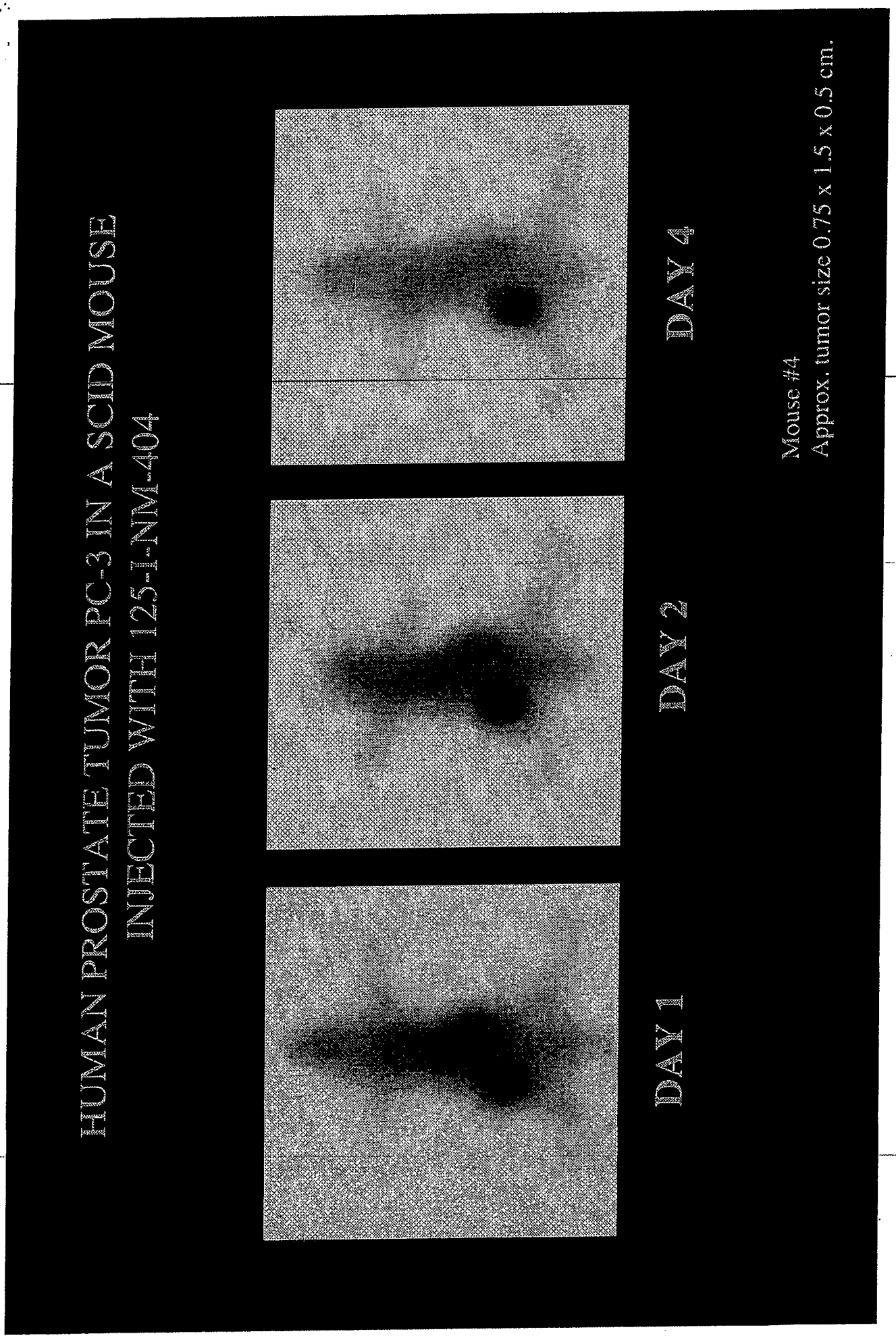

HUMAN PROSTATE TUMOR PC-3 IN A SCID MOUSE
INJECTED WITH 125-I-NM404
DAY 1
DAY 2
DAY 4
Mouse #4
Approx. tumor size 0.75 x 1.5 x 0.5 cm.

FIGURE 2a

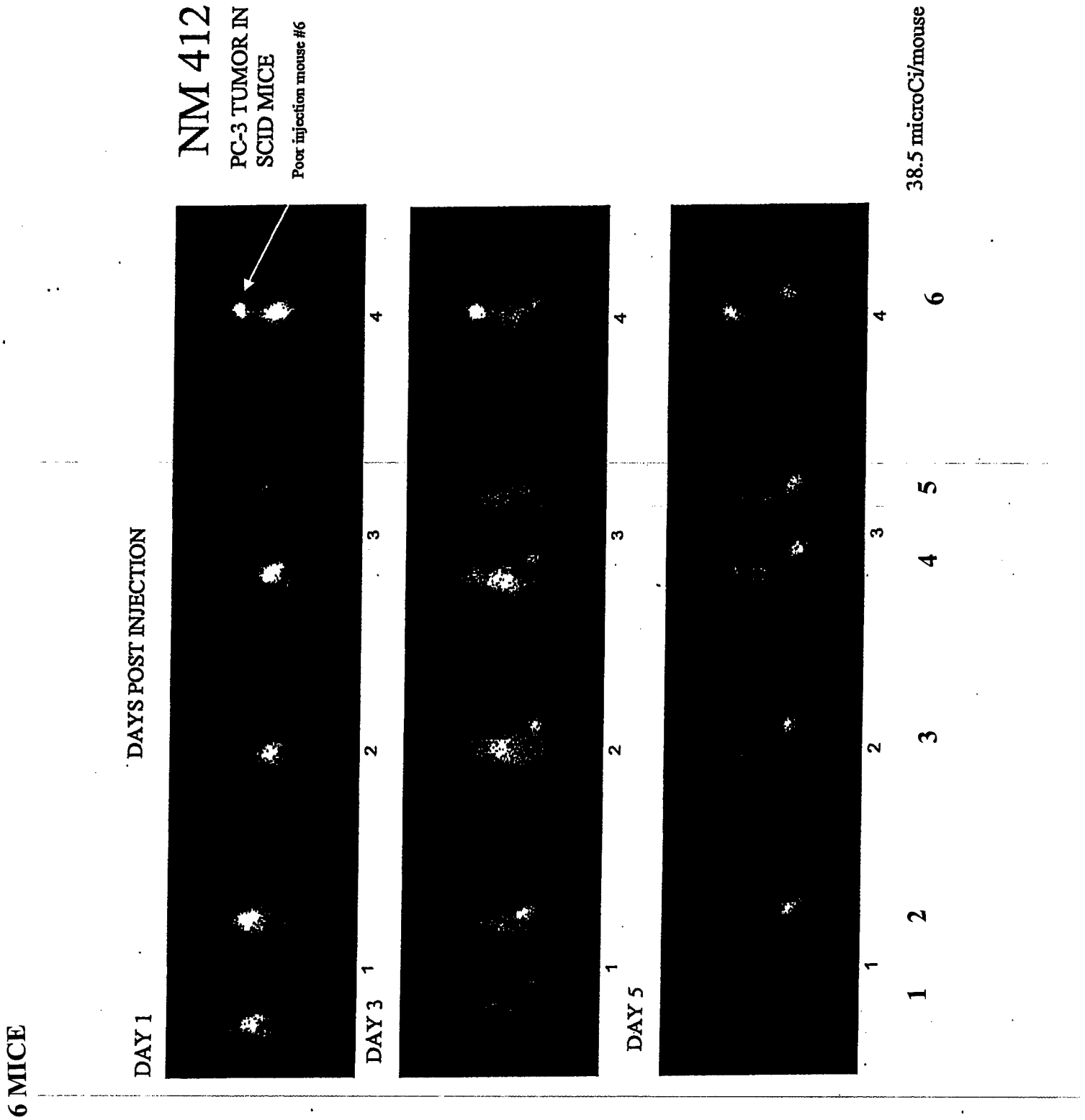
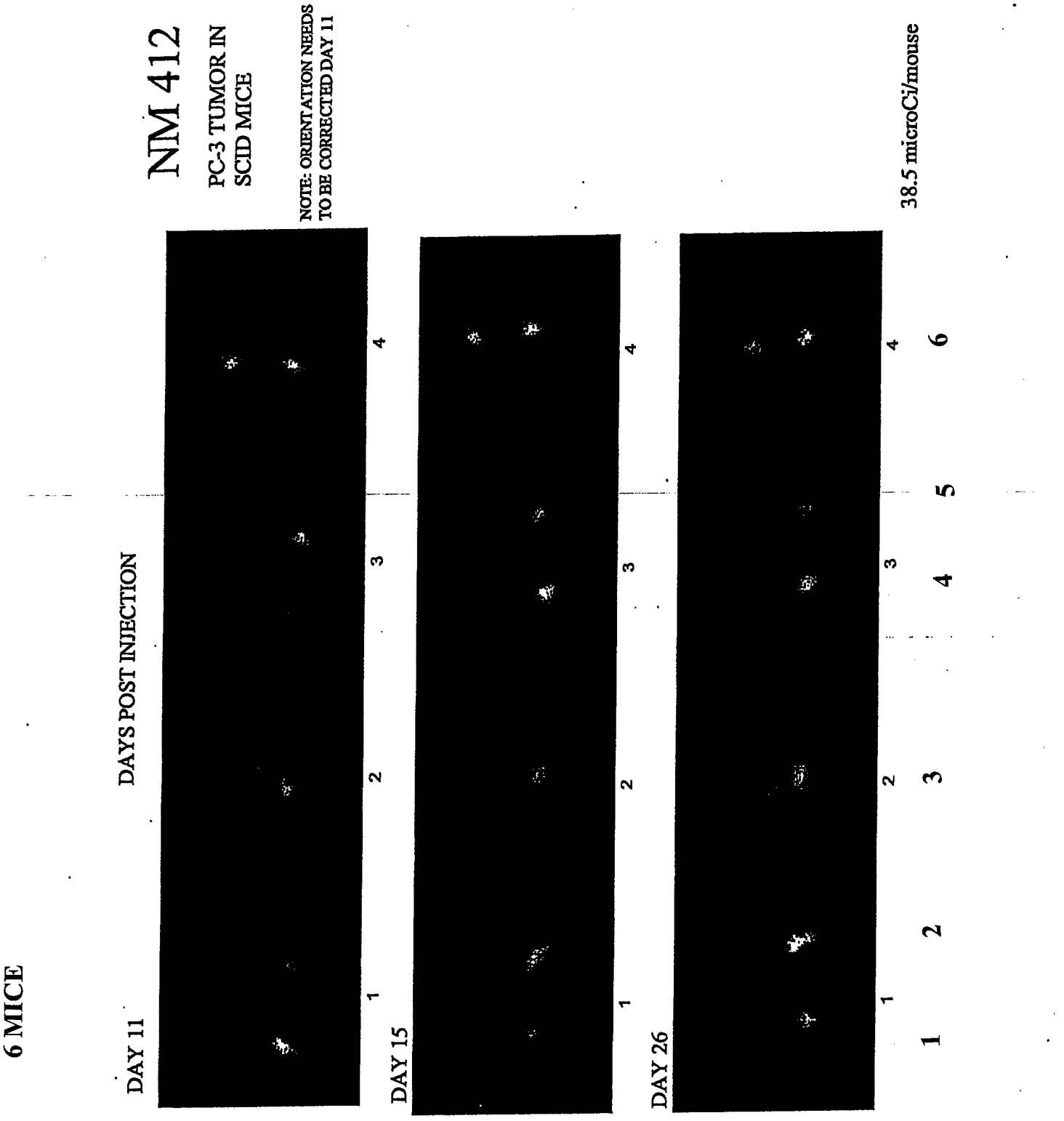

NM 412
PC-3 TUMOR IN SCID MICE
Poor injection mouse #6
38.5 microCi/mouse
6 MICE
DAYS POST INJECTION
DAY 1
DAY 3
DAY 5
1 2 3 4 5 6

FIGURE 2b


NM 412
PC-3 TUMOR IN SCID MICE
NOTE: ORIENTATION NEEDS TO BE CORRECTED DAY 11
6 MICE
DAYS POST INJECTION
DAY 11
DAY 15
DAY 26
38.5 microCi/mouse
1 2 3 4 5 6

TLC OF BUTANOL EXTRACTS OF SCID MICE TUMORS FROM SCID MICE WITH PC-3 PROSTATE CANCER XENOGRAFTS INJECTED WITH 125I-NM-404

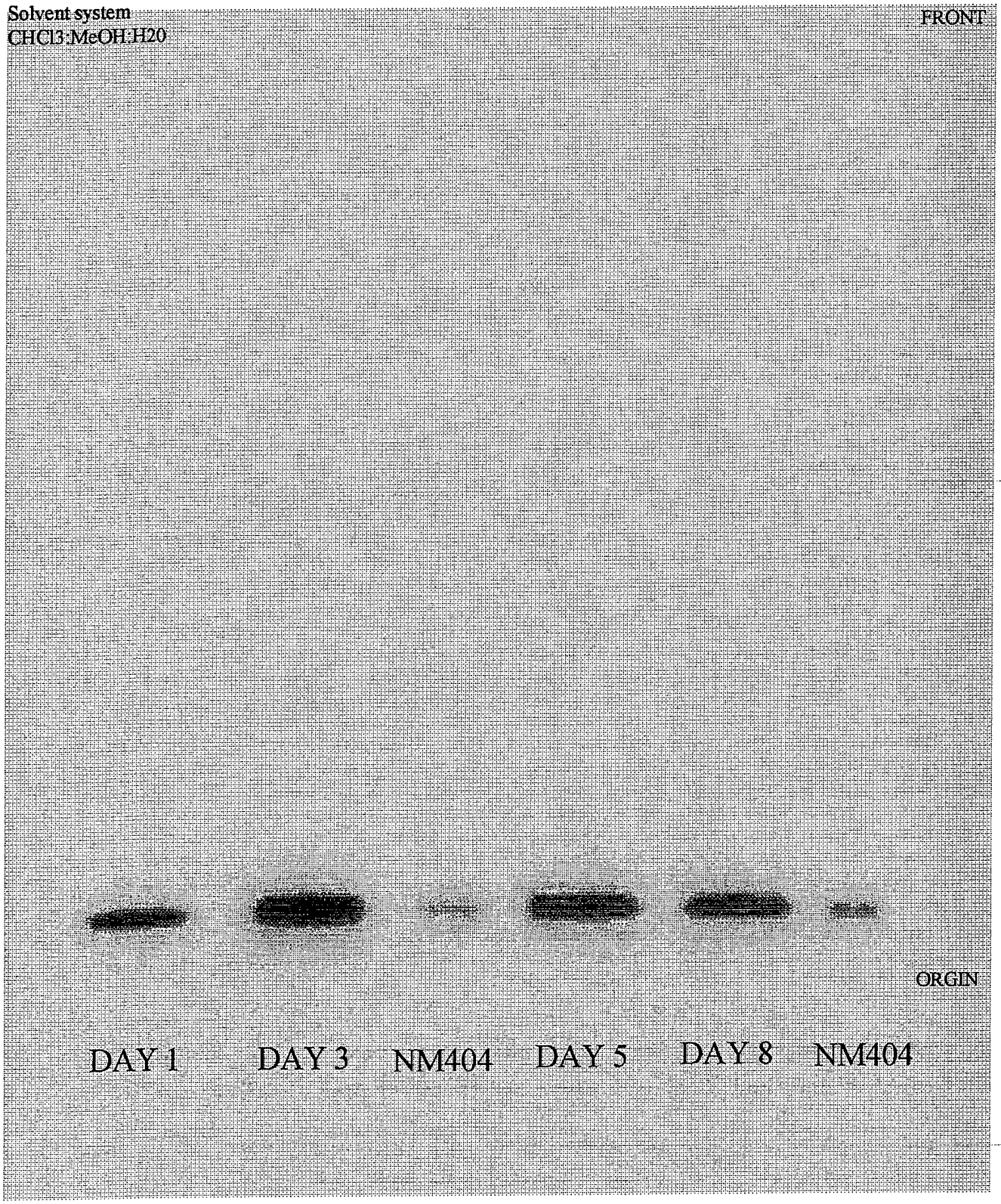
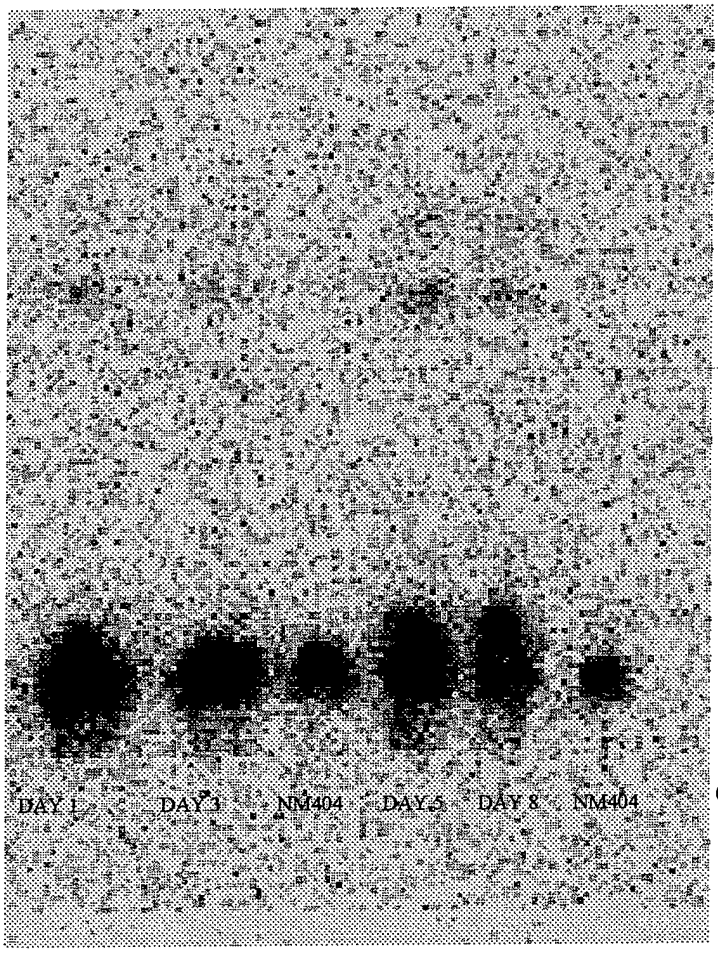


FIGURE 4

TLC OF BUTANOL EXTRACTS OF SCID MICE PLASMA FROM SCID MICE WITH PC-3 PROSTATE CANCER XENOGRAFTS INJECTED WITH 125I-NM-404

SOLVENT SYSTEM
CHCl3: MeOH: H2O



BIODISTRIBUTION STUDIES AND RADIATION DOSIMETRY: In order to provide the necessary data for dosimetry calculations, it was necessary to conduct biodistribution studies of both NM-404 and NM-412 in male Sprague-Dawley rats. Animals were injected with NM-404 as described above and the tissues were analyzed for radioactivity after 1, 6, 24, 72 hours and 7 and 10 days. The biodistribution results, dosimetry and residence time calculations are shown in Appendix 2. Similarly the data for NM-412 are shown in Appendix 3. On the basis of the data obtained for NM-404, our health physicist predicted that NM-404 labeled with iodine-131 could be safely administered to humans along with thyroid blocking with KI solution at a dose not to exceed 2 mCi.

ASSESSMENT OF ACUTE TOXICITY IN ANIMALS: Dr. Paul Kostyniak and his group at the Toxicology Center of the University of New York at Buffalo examined both stable NM-404 and NM-412 for acute toxicity. These studies were conducted in rats and rabbits. One group of each species was administered the test agent while another group was administered the vehicle only. The final report for NM-404 is provided as Appendix 4. The report for NM-412 was essentially the same and can be furnished if desired. Dr. Kostyniak reported an absence of toxic manifestations for both agents at a dose that was greater than 1000 times that which would be administered to humans.

APPLICATION FOR APPROVAL FOR A PRELIMINARY CLINICAL EVALUATION OF NM-404 IN PROSTATE CANCER PATIENTS: In order to undertake a preliminary pharmacokinetic appraisal of NM-404 in prostate cancer patients, it was necessary to obtain approval from various committees in addition to the Department of Defense. With the departure of Dr. Richard Wahl, this preliminary study would be under the direction of Dr. Milton D. Gross, Professor of Radiology and Internal Medicine.
Internally applications are required for the Clinical Research Center (CRC) of the University Hospital, the University's Institutional Review Board for Human Subject Research (IRB), and the Radioactive Drug Research Committee (RDRC). The latter committee serves as an arm of the Food and Drug Administration and oversees the use of radioactive tracers in human studies. A change in policy by this committee made it necessary for us to file an Investigational New Drug (I.N.D.) application with the F.D.A. prior to review by the RDRC. Such an application was submitted to the F.D.A. on May 29, 2001. In July 2001, Dr. Gross received a letter from the F.D.A. indicating that they had completed their 30-day safety review and concluded that he could proceed with the proposed clinical investigation, Appendix 5.

ASSESSMENT OF TOXICITY IN HUMANS: RDRC requires that a preliminary toxicity evaluation of the stable tracer be conducted in five normal volunteers at a dose 5 to 10 times the anticipated imaging dose to be administered to cancer patients. As requested, the Volunteer Registry Data Sheets for the five normal volunteers was sent to Michelle M. Von Reichenbach of the Medical Research Materiel Command on December 7, 2001 (see attached).

7. KEY RESEARCH ACCOMPLISHMENT

- Accomplished the synthesis of NM-404 and NM-412 in sufficient quantity to accommodate future research objectives.
- Developed an improved method for radiolabeling NM-404 and NM-412 with radioiodine, which led to products with substantially higher specific activity.
- By using SCID mice bearing the PC-3 prostate tumor, we were able to demonstrate by tissue analysis the remarkable ability of both NM-404 and NM-412 to concentrate in the tumor.
- Confirmed the tumor avidity of NM-404 and NM-412 by gamma camera scintigraphy. The excellent tumor targeting and the excellent images obtained with NM-404 underscored its potential for human tumor diagnosis.
- Completed the necessary animal studies to permit radiation dosimetric calculations for both NM-404 and NM-412.
- Successfully demonstrated that appropriately formulated NM-404 and NM-412 lacked toxicity in animals at a dose over one thousand times the anticipated dose to humans.
- Compiled and submitted and Investigational New Drug Application to the Food and Drug Administration for the study of NM-404 in human subjects.
- Received a positive response from the University of Michigan General Clinical Research Center for the purpose of conducting patient studies with NM-404 in the clinical facilities.
- Submitted applications to the University's Institutional Review Board and the Radioactive Drug Research Committee for approval to conduct a preliminary pharmacokinetic evaluation of radioiodinated NM-404 in patients with prostate cancer (pending).

8. REPORTABLE OUTCOMES:

Manuscripts, Abstracts and Presentations:

Zasadny KR, Longino MA, Fisher SJ, Counsell RE and Wahl RL. Predicted Dosimetry for 131-I NM-404, A Phospholipid Ether Agent for Tumor Imaging and Possible Therapy. *J Nucl Med* 40:39P, 1999.

Counsell RE, Longino MA, Pinchuk AN, Skinner RWS, Fisher SJ, Van Dort ME, Pienta KF and Wahl RL. Synthesis and Evaluation of a Radioiodinated Phospholipid Ether Analog (NM-404) for Diagnostic Imaging of Prostate Cancer. *Isotope Production and Applications in the 21st Century*, NR Stevenson, ed., World Scientific, Singapore, pp163-166, 2000.

Counsell RE, Longino MA, Pinchuk AN, Van Dort ME, Fisher SJ, Skinner RWS, Zasadny KR and Wahl RL. Radioiodinated Phospholipid Ethers and Analogs as Tumor Imaging Agents. Fourth International Symposium on Radiohalogens, Whistler, B.C., Canada, September 9-13, 2000.

Funding Applied for Based on Work Supported by this Award:

With the depletion of funds on the present award, application was made to several organizations to support the clinical phases of the study. They were as follows:

- Office of the Vice President for Research. In October, 2001 submitted an application entitled "Clinical evaluation of NM-404 for the Noninvasive Imaging of Prostate Cancer". Requested $14,252 and $8,000 were awarded.

- 2001 CaP CURE: In October, 2001 submitted an application with the same title as above was submitted. Requested $100,000, which was denied.

9. CONCLUSIONS:

Progress toward our stated goals was excellent for the preclinical phases of our project. Studies with tumor-bearing animals clearly demonstrated the remarkable ability of NM-404 and NM-412 to selectively accumulate in the tumor. Such successful targeting in animals clearly demonstrated the potential of such agents for the noninvasive imaging of tumors in humans. Moreover, the high level of tumor uptake shown for NM-404 suggests its potential as a possible agent for therapy. The high specific activity that we have achieved in our radioiodination procedure means that the actual amount of drug that would be administered in a clinical dose is extremely small, and toxicologic studies have demonstrated these agents to be devoid of toxicity at doses much higher than those anticipated for humans.

Three factors played a key role in hampering the translation of our preclinical results to the clinic. The initial setback occurred when Dr. Richard Wahl, the original P.I. for the project, left to become Professor and Chairman of the Division of Nuclear Medicine at Johns Hopkins Medical School. Upon Dr. Wahl's departure, Dr. Counsell became overall Principle Investigator in November 2000. Fortunately, Dr. Milton Gross, Professor of Radiology and Internal Medicine, who had been associated with our research for many years was available to assume responsibility for the clinical phases of the project. A second setback occurred when the RDRC changed their previous policy to require approval from the F.D.A. for an Investigational New Drug. Obviously, if we had known this in advance, we would have been less optimistic with our time lines. Nonetheless, our application was approved by the F.D.A. and efforts turned to getting approval for the clinical studies from the various internal Hospital and University committees and the Department of Defense. Moreover, since grant funds were becoming depleted, application was made to other agencies to support the proposed preliminary clinical study in prostate cancer patients (see section **8**). With such support and our view that the D.O.D. wished to see us complete what we had outlined in our proposal, we requested "no cost time extensions" from D.O.D. which were approved. The final surprise came in the form of an e-mail on 5/30/02 from Ms. Kathy A. Witman, D.O.D. Contract Specialist, informing us to not proceed with the patient study as it "involved more than minimal risk". An adequate explanation for this decision was not provided. Accordingly, we are submitting our final report and wish to thank the Department of Defense for their support of our research. Our gratitude will be appropriately indicated on all future publications citing this research.

10. APPENDICES:

Appendix 1: Synthesis of NM-404 and NM-412
Appendix 2: Biodistribution of NM-404 in Sprague-Dawley Rats and Dosimetry
Appendix 3: Biodistribution of NM-412 in Sprague-Dawley Rats and Dosimetry
Appendix 4: Acute Toxicology of NM-404 in Rats and Rabbits
Appendix 5: Letter from Food and Drug Administration and I.N.D. Number
Appendix 6: Presentations and Publications

Personnel Receiving Pay for the Research Effort:

Raymond E. Counsell, PhD
Richard L. Wahl, MD
Marc A. Longino, PhD
Marcian Van Dort, PhD
Denise Regan, BS
Susan Korenchuk, BS
Joanna Hooten

Appendix 1: Synthesis of NM-404 and NM-412

SYNTHESIS OF NM-404 (15) AND NM-412 (30)

^{1}H-NMR spectra were recorded on an AM-360 Bruker spectrometer using Me_4Si as an internal standard. Melting points were measured using a Melt-Temp apparatus and are uncorrected. Thin-layer chromatography was performed using DC-Alufolien Kieselgel 60 F plates (E. Merck, Darmstadt, Germany). Visualization was achieved by UV light and/or charring after spraying with 5 % H_2SO_4 in ethanol. For flash chromatography, silica gel 32-63 mkm (Fisher Scientific) was used. All chemicals were obtained from Aldrich Chemical Co. (Milwaukee, WI) except anhydrous trimethylamine which was from Fluka. Elemental analysis results were within ±0.4% of the theoretical values.

p-Iodobenzyl alcohol (2)

To a solution of p-iodobenzoic acid (5g, 20 mmol) in anhydrous THF (5 ml) was added BH_3-THF complex (40 ml of 1.0 M solution, 40 mmol) dropwise at 0°C under an argon atmosphere. The reaction mixture was allowed to warm to room temperature and stirred for 5 h, then was cooled again to 0°C and quenched with H_2O. Ethyl acetate and additional water were added. The organic layer was separated, washed with water and dried (Na_2SO_4). Evaporation of solvent gave a white solid (4.7 g, 100 %) which was used in the next step without purification. Analytical sample was crystallized from hexane, mp 71-73°C. ^{1}H-NMR ($CDCl_3$): 7.69 and 7.12 (two dt, 2H each, Ar-H); 4.65 (s, 2H, CH_2).

p-Iodobenzyl iodide (3)

To a solution of p-iodobenzyl alcohol **2** (4.7 g, 20 mmol) and sodium iodide (6 g, 40 mmol; dried at t°>100°C) in anhydrous acetonitrile (40 ml) was slowly added chlorotrimethylsilane (5 ml, 40 mmol) with stirring. The mixture was stirred at room temperature for 1.5, then diluted with ether and washed successively with water, $Na_2S_2O_3$ solution and brine. The organic phase was dried over Na_2SO_4. Silica gel chromatography with hexane-ethyl acetate (gradient from 100:0 to 100:5) gave the product as yellowish crystals (6.56 g, 95%), mp 83-84°C (long needles from hexane). ^{1}H-NMR ($CDCl_3$): 7.62 and 7.12 (two dt, 2H each, Ar-H); 4.38 (s, 2H, CH_2).

Tetrahydro-2-(11-bromoundecyloxy)-2*H*-pyran (4)

A solution of 11-bromoundecanol (6 g, 24 mmol) and dihydropyran (3.3 ml, 36 mmol) in methylene chloride (20 ml) containing pyridinium p-toluenesulfonate (600 mg, 2.4 mmol) was stirred at room temperature for 5 h. The solution was diluted with hexane, washed with water and dried (Na_2SO_4). Chromatography in hexane-ether (150:5) afforded the product (7.93 g, 99%) as a clear oil. ^{1}H-NMR ($CDCl_3$): 4.59-4.56 (m, 1H, anomeric 2-CH of THP), 3.91-3.84 (m, 1H, 6-CH_{eq} of THP), 3.73 (dt, 1H, CH_AH_BOTHP), 3.54-
3.46 (m, 1H, 6-CH_{ax} of THP), 3.41 (t, 2H, CH_2Br), 3.39 (dt, 1H, CH_AH_BOTHP), 1.85 (quintet, 2H, $BrCH_2CH_2$), 1.82-1.67 (m, 2H, THP), 1.64-1.40 (m, 6H, CH_2CH_2OTHP and 4H of THP), 1.40-1.20 (m, 16H, $(CH_2)_8$).

Tetrahydro-2-(3-bromopropyloxy)-2*H*-pyran (8)

Following the procedure described for the preparation of **4**, the title compound was obtained from 3-bromopropanol (1 g, 7.2 mmol) in 98 % yield as a clear oil. ^{1}H-NMR ($CDCl_3$): 4.62-4.59 (m, 1H, anomeric 2-CH of THP), 3.90-3.83 (m, 1H, 6-CH_{eq} of THP), 3.88 (dt, 1H, CH_AH_BOTHP), 3.56-3.48 (m, 1H, 6-CH_{ax} of THP), 3.54 (t, 2H, $BrCH_2$), 3.52 (dt, 1H, CH_AH_BOTHP), 2.14 (quintet, 2H, $BrCH_2CH_2$), 1.84-1.66 (m, 2H, THP), 1.64-1.49 (m, 6H, 4H, THP).

Tetrahydro-2-(6-bromohexadecyloxy)-2*H*-pyran (11)

This compound was prepared from 6-bromohexanol (1.38 g, 7.62 mmol) in 92 % yield (clear oil) according to the general procedure described for **4**. ^{1}H-NMR ($CDCl_3$): 4.59-4.56 (m, 1H, anomeric 2-CH of THP), 3.91-3.86 (m, 1H, 6-CH_{eq} of THP), 3.74 (dt, 1H, C*H*$_A$H$_B$OTHP), 3.55-3.47 (m, 1H, 6-CH_{ax} of THP), 3.41 (t, 2H, CH_2Br), 3.39 (dt, 1H, CH$_A$*H*$_B$OTHP), 1.87 (quintet, 2H, BrCH_2*C*H_2), 1.83-1.67 (m, 2H, THP), 1.64-1.37 (m, 10H, (C*H*$_2$)$_3$$CH_2$OTHP and 4H of THP).

Tetrahydro-2-(12-p-iodophenyldodecyloxy)-2*H*-pyran (5)

Grignard reagent was prepared from bromide **4** (2.715 g, 8.1 mmol) and approx. three-times excess of magnesium powder. Magnesium powder was suspended in THF (2.5 ml) and dibromoethane (0.05 ml) was added for activation. After 10 min, the THF solution was withdrawn by syringe and replaced with 5 ml of fresh THF. A solution of the bromide **4** in THF (20 ml) was added dropwise over 1h at room temperature. Then, a solution of Grignard reagent was transferred into a round-bottom flask via canula and cooled to -78^{o}C. A solution of Li_2CuCl_4 in THF (0.5 ml of 0.12 mmol/ml solution, 0.06 mmol) was added to the Grignard reagent with stirring followed by a solution of p-iodobenzyl iodide **4** (3.2 g, 9.32 mmol) in THF (20 ml). The reaction mixture was allowed to warm to room temperature during 2 h and stirring was continued for additional 12 h. The reaction mixture was quenched with ammonium chloride solution and extracted with ethyl acetate. The extract was washed with water and dried (Na_2SO_4). The solvent was removed in vacuo and the residue was chromatographed on silica gel, first eluting with hexane-chloroform (8:2), then with hexane-THF (150:3) to give a clear oil (2.37 g, 62 %) ^{1}H-NMR ($CDCl_3$): 7.58 and 6.92 (two dt, 2H each, Ar-H), 4.59-4.57 (m, 1H, anomeric 2-CH of THP), 3.91-3.84 (m, 1H, 6-CH_{eq} of THP), 3.73 (dt, 1H, C*H*$_A$H$_B$OTHP), 3.54-3.46 (m, 1H, 6-CH_{ax} of THP), 3.38 (dt, 1H, CH$_A$*H*$_B$OTHP), 2.55 (t, 2H, Ar*CH*$_2$), 1.82-1.67 (m, 2H, THP), 1.64-1.45 (m, 8H, Ar-CH_2*C*H_2, *C*$H_2$$CH_2$OTHP and 4H of THP), 1.40-1.20 (m, 16H, $(CH_2)_8$).

12-p-(Iodophenyl)dodecanol (6)

A solution of THP ether **5** (4.068 g, 8.62 mmol) and pyridinium p-toluenesulfonate (216 mg, 0.86 mmol) in ethanol (20 ml) was stirred at 50^{o}C for 3 h until TLC showed no starting material. The reaction mixture was diluted with water and extracted with ethyl acetate, washed with water, dried (Na_2SO_4) and evaporated. Silica gel chromatography of the residue in hexane-ethyl acetate (85:15) gave the product (3.01 g, 90 %) as a white solid, mp In cases when contamination by the aliphatic alcohol derived from Grignard reagent was revealed by NMR, the product was crystallized from hexane at 15^{o}C (for alcohols **10** and **13** at 0^{o}C). ^{1}H-NMR ($CDCl_3$): 7.58 and 6.92 (two dt, 2H each, Ar-H), 3.64 (t, 2H, CH_2OH), 1.52-1.60 (m, 4H, ArCH_2*C*H_2 and *C*$H_2$$CH_2$OH), 1.35-1.22 (m, 16H, $(CH_2)_8$).

12-p-(Iodophenyl)dodecyl tosylate (7)

A solution of 12-p-(iodophenyl)dodecanol **6** (2.01 g, 5.18 mmol), tosyl chloride (1.09 g, 5.7 mmol) and N,N-dimethylaminopyridine (0.72 g, 5.9 mmol) in dichloromethane (15 ml) was stirred for 6 h. The clear reaction mixture was diluted with 10 ml of hexane and carefully poured directly onto the top of a silica gel column. The column was eluted with hexane-chloroform (1:1), then with chloroform to give the tosylate (2.69 g, 96 %) as a slightly colored solid, mp 39-41^{o}C. ^{1}H-NMR ($CDCl_3$): 7.58 and 6.92 (two dt, 2H each, IC_6H_4), 7.79 and 7.34 (two dt, 2H each, CH_3C_6*H*$_4$$SO_3$), 4.02 (t, 2H, C*H*$_2$OTs), 2.53 (t, 2H, Ar-C*H*$_2$), 2.44 (s, 3H, C*H*$_3$$C_6H_4SO_3$), 1.62-1.50 (m, 4H, Ar-$CH_2$*C*$H_2$ and *C*$H_2$$CH_2$OTs), 1.32-1.18 (m, 16H, $(CH_2)_8$).

Tetrahydro-2-(18-p-iodophenyloctadecyloxy)-2*H*-pyran (12)

Magnesium powder (370 mg, 15.4 mmol) in THF (2.5 ml) was activated by addition of 1.2-dibromoethane (0.03 ml) and stirring for 10 min. After the reaction with dibromoethane had ceased, the solution was removed via syringe and replaced with fresh THF (5 ml). This procedure was followed by the addition of bromide **8** (1.373 g, 5.18 mmol) in THF (10 ml) over 1h at room temperature. When all the halide had been added, stirring was continued for additional 15 min whereupon the small aliquot was hydrolyzed and analyzed by TLC in hexane-THF (150:6) which revealed no presence of starting bromide. The Grignard reagent was transferred to a round bottom flask and cooled to -78°C. The organometallic reagent was stirred for 10 min at this temperature before a solution of Li_2CuCl_4 in THF (0.5 ml of 0.077 mmol/ml solution, 0.0385 mmol) was added followed by 12-p-(iodophenyl)dodecyl tosylate **7** (2.69 g, 4.96 mmol) dissolved in THF (10 ml). The reaction mixture was allowed to gradually warm to room temperature for 3 h and was kept at this temperature for additional 12 h before a saturated ammonium chloride solution was added to quench the reaction. The mixture was extracted with hexane, washed with water and dried (Na_2SO_4). The solvent was removed in vacuo and silica gel chromatography with hexane-THF (150:3) gave the product (1.77 g, 64 %) as a white wax, mp 26-27°C. ^{1}H-NMR ($CDCl_3$): 7.58 and 6.92 (two dt, 2H each, Ar-H), 4.59-4.57 (m, 1H, anomeric 2-CH of THP), 3.91-3.84 (m, 1H, 6-CH_{eq} of THP), 3.73 (dt, 1H, CH_AH_BOTHP), 3.54-3.46 (m, 1H, 6-CH_{ax} of THP), 3.38 (dt, 1H, CH_AH_BOTHP), 2.55 (t, 2H, ArCH_2), 1.82-1.67 (m, 2H, THP), 1.64-1.45 (m, 8H, Ar-CH_2CH_2 , CH_2CH_2OTHP and 4H of THP), 1.39-1.22 (m, 28H, $(CH_2)_{14}$).

Tetrahydro-2-(15-p-iodophenylpentadecyloxy)-2*H*-pyran (9)

This compound was obtained in a manner analogous to that of compound **12** from tosylate **7** (300 mg, 0.55 mmol) and bromide **8** (136 mg, 0.61 mmol). Silica gel chromatography as before gave the product (165 mg, 52 %). ^{1}H-NMR ($CDCl_3$): 7.58 and 6.92 (two dt, 2H each, Ar-H), 4.59-4.57 (m, 1H, anomeric 2-CH of THP), 3.91-3.84 (m, 1H, 6-CH_{eq} of THP), 3.73 (dt, 1H, CH_AH_BOTHP), 3.54-3.46 (m, 1H, 6-CH_{ax} of THP), 3.38 (dt, 1H, CH_AH_BOTHP), 2.55 (t, 2H, ArCH_2), 1.82-1.67 (m, 2H, THP), 1.64-1.45 (m, 8H, Ar-CH_2CH_2 , CH_2CH_2OTHP and 4H of THP), 1.39-1.22 (m, 22H, $(CH_2)_{11}$).

15-(p-Iodophenyl)pentadecanol (10)

Compound **9** (150 mg g, 0.26 mmol) was converted to the desired product by the procedure described for **6**. Alcohol **10** was obtained with a yield of 91 %, ^{1}H-NMR ($CDCl_3$): 7.57 and 6.92 (two dt, 2H each, Ar-H),3.64 (t, 2H, CH_2OH), 2.54 (t, 2H, Ar-CH_2), 1.60-1.55 (m, 4H, Ar-CH_2CH_2 and CH_2CH_2OH), 1.35-1.22 (m, 22H, $(CH_2)_{11}$).

18-(p-Iodophenyl)octadecanol (13)

By the procedure described for **6**, THP ether **12** (1.4 g, 2.5 mmol) was converted to the desired product **13** with 85 % yield, mp 64-67°C (from hexane). ^{1}H-NMR ($CDCl_3$): 7.58 and 6.92 (two dt, 2H each, Ar-H), 3.64 (t, 2H, CH_2OH), 2.54 (t, 2H, Ar-CH_2), 1.60-1.55 (m, 4H, Ar-CH_2CH_2 and CH_2CH_2OH), 1.35-1.20 (m 28H, $(CH_2)_{14}$).

18-(p-Iodophenyl)octadecyl methanesulfonate (17)

To a solution of 18-(p-iodophenyl)octadecanol **13** (150 mg, 0.317 mmol) and triethylamine (0.07 ml, 0.48 mmol) in methylene chloride (2 ml) was added methane sulfonyl chloride (0.03 ml, 0.38 mmol) at 0°C.

Stirring was continued for 40 min whereupon the reaction mixture was quenched by addition of water. The reaction mixture was diluted with chloroform and washed several times with $NaHCO_3$ solution and water. The chloroform layer was dried (Na_2SO_4) and and the solvent was removed in vacuo. The residue was chromatographed with hexane - ethyl acetate (9:1). This afforded pure **17** (142 mg; 82 %), mp 61-62°C (from ethanol). ^{1}H-NMR ($CDCl_3$): 7.58 and 6.92 (two dt, 2H each, Ar-H), 4.22 (t, 2H, C*H*$_2$OMs), 3.00 (s, 3H, CH_3S), 2.53 (t, 2H, Ar-C*H*$_2$), 1.75 (quintet, 2H, C*H*$_2$CH$_2$OMs), 1.60-1.5 (m, 2H, Ar-CH$_2$C*H*$_2$), 1.40- 1.20 (m, 28H, $(CH_2)_{14}$).

15-(p-Iodophenyl)pentadecyl methanesulfonate (16)

By the above procedure, alcohol **10** (150 mg, 0.35 mmol) was converted to the desired product **16** (163 mg, 92 %), mp
^{1}H-NMR ($CDCl_3$): 7.58 and 6.92 (two dt, 2H each, Ar-H), 4.22 (t, 2H, C*H*$_2$OMs), 3.00 (s, 3H, CH_3S), 2.53 (t, 2H, Ar-CH_2), 1.75 (quintet, 2H, C*H*$_2$CH$_2$OMs), 1.60-1.50 (m, 2H, Ar-CH$_2$C*H*$_2$), 1.40- 1.20 (m, 22H, $(CH_2)_{11}$).

1-*O*-[18-(p-Iodophenyl)octadecyl]-3-*O*-benzyl-1,3-propanediol (22)

To a solution of 3-benzyloxypropanol **18** (0.03 ml; 0.18 mmol) and 18-(p-iodophenyl) octadecyl methanesulfonate **17** (66 mg; 0.12 mmol) in dimethylformamide (3 ml) was added sodium hydride (8 mg of 60 % suspension in oil; 0.2 mmol) at room temperature. The reaction mixture was stirred for 12 hr, quenched with water and extracted with ethyl acetate. The extract was washed with brine, dried (Na_2SO_4) and the solvent was removed in vacuo. Column chromatography with hexane-ethyl acetate (gradient from 95:5 to 85:15) afforded **22** (60 mg; 81 %). ^{1}H-NMR ($CDCl_3$): 7.58 and 6.92 (two dt, 2H each, Ar-H), 7.36-7.30 (m, 5H, C_6H_5), 4.50 (s, 2H, PhC*H*$_2$), 3.57 (t, 2H, alkyl-OC*H*$_2$(CH$_2$)$_2$O), 3.52 (t, 2H, C*H*$_2$OBn), 3.39 (t, 2H, C*H*$_2$O(CH$_2$)$_3$)O), 2.53 (t, 2H, Ar-CH_2), 1.90 (quintet, 2H, OCH$_2$C*H*$_2$CH$_2$O), 1.65-1.55 (m, 4H, Ar-CH$_2$C*H*$_2$ and C*H*$_2$CH$_2$O(CH$_2$)$_3$O), 1.35-1.20 (m, 28H, $(CH_2)_{14}$).

1-*O*-[15-(p-Iodophenyl)pentadecyl]-3-*O*-benzyl-1,3-propanediol (20)

This compound was synthesized as described above from mesylate **16** (85 mg, 0.17 mmol) and 3-benzyloxypropanol **18** (0.036 ml; 0.23 mmol). The compound **20** was obtained in a yield of 79 % (76 mg) after chromatographic purification. ^{1}H-NMR ($CDCl_3$): 7.58 and 6.92 (two dt, 2H each, Ar-H), 7.36-7.30 (m, 5H, C_6H_5), 4.50 (s, 2H, PhC*H*$_2$), 3.57 (t, 2H, alkyl-OC*H*$_2$(CH$_2$)$_2$O), 3.52 (t, 2H, C*H*$_2$OBn), 3.39 (t, 2H, C*H*$_2$O(CH$_2$)$_3$)O), 2.53 (t, 2H, Ar-CH_2), 1.89 (quintet, 2H, OCH$_2$C*H*$_2$CH$_2$O), 1.65-1.55 (m, 4H, Ar-CH$_2$C*H*$_2$ and C*H*$_2$CH$_2$O(CH$_2$)$_3$O), 1.40-1.20 (m, 22H, $(CH_2)_{11}$).

1-*O*-[15-(p-Iodophenyl)pentadecyl]-2-*O*-methyl-3-*O*-benzyl-*rac*-glycerol (21)

This compound was synthesized as described for **22** from mesylate **16** (92 mg, 0.18 mmol) and 1-*O*-benzyl-2-*O*-methyl-*rac*-glycerol **19** (43 mg, 0.22 mmol) to give 75 mg (68 %) of the product. ^{1}H-NMR ($CDCl_3$): 7.58 and 6.92 (two dt, 2H each, Ar-H), 7.35-7.25 (m, 5H, C_6H_5), 4.55 (s, 2H, PhC*H*$_2$), 3.60-3.50 (m, 5H, CH$_2$CHCH$_2$), 3.45 (s, 3H, OCH_3), 3.42 (t, 2H, C*H*$_2$OCH$_2$CH), 2.53 (t, 2H, IC$_6$H$_4$C*H*$_2$), 1.60-1.50 (m, 4H, ArCH$_2$C*H*$_2$ and C*H*$_2$CH$_2$O), 1.35-1.20 (m, 22H, $(CH_2)_{11}$).

1-*O*-[18-(p-Iodophenyl)octadecyl]-2-*O*-methyl-3-*O*-benzyl-*rac*-glycerol (23)

This compound was synthesized as described for **22** from mesylate **17** (67 mg, 0.12 mmol) and 1-*O*-benzyl-2-*O*-methyl-*rac*-glycerol **19** (36 mg, 0.18 mmol) to give 62 mg (78 %) of the product. ^{1}H-NMR ($CDCl_3$): 7.58 and 6.92 (two dt, 2H each, Ar-H), 7.35-7.25 (m, 5H, C_6H_5), 4.55 (s, 2H, PhCH_2), 3.60-3.50 (m, 5H, CH_2CHCH_2), 3.45 (s, 3H, OCH_3), 3.42 (t, 2H, CH_2OCH_2CH), 2.53 (t, 2H, $IC_6H_4CH_2$), 1.60-1.50 (m, 4H, $ArCH_2CH_2$ and CH_2CH_2O), 1.35-1.20 (m, 28H, $(CH_2)_{14}$).

1-*O*-[18-(p-Iodophenyl)octadecyl]-1,3-propanediol (26)

To a solution of benzyl ether **22** (413 mg, 0.66 mmol) and anisole (0.36 ml, 3.33 mmol) in methylene chloride (10 ml) was added powdered aluminum chloride (353 mg, 2.66 mmol) at room temperature. Stirring was continued for 2 h. The reaction mixture was quenched by dilution with 1N HCl, and aqueous layer was extracted with ethyl acetate. The organic layer was washed with NaHCO3 solution, dried (Na_2SO_4) and evaporated. The remaining residue was chromatographed with hexane-ethyl acetate (gradient from 95:5 to 80:20) to give the product (301 mg, 85 %). ^{1}H-NMR ($CDCl_3$): 7.58 and 6.92 (two dt, 2H each, Ar-H), 3.78 (q, 2H, CH_2OH), 3.62 (t, 2H, $OCH_2CH_2CH_2OH$), 3.42 (t, 2H, $CH_2O(CH_2)_3OH$), 2.55 (t, 2H, Ar-CH_2, 2H), 1.83 (quintet, 2H, $OCH_2CH_2CH_2OH$), 1.60-1.50 (m, 4H, Ar-CH_2CH_2 and CH_2CH_2O), 1.35-1.20 (m, 28H, $(CH_2)_{14}$).

1-*O*-[15-(p-Iodophenyl)pentadecyl]-1,3-propanediol (24)

By the above procedure, compound **20** (76 mg, 0.13 mmol) was converted to the alcohol **24** (60 mg, 94 %). ^{1}H-NMR ($CDCl_3$): 7.58 and 6.92 (two dt, 2H each, Ar-H), 3.78 (q, 2H, CH_2OH), 3.62 (t, 2H, $OCH_2CH_2CH_2OH$), 3.42 (t, 2H, $CH_2O(CH_2)_3OH$), 2.55 (t, 2H, Ar-CH_2, 2H), 1.83 (quintet, 2H, $OCH_2CH_2CH_2OH$), 1.60-1.45 (m, 4H, Ar-CH_2CH_2 and CH_2CH_2O), 1.35-1.20 (m, 22H, $(CH_2)_{11}$).

1-*O*-[18-(p-Iodophenyl)pentadecyl]-2-*O*-methyl-*rac*-glycerol (25)

Compound **21** (75 mg, 0.12 mmol) was converted to the desired alcohol, **25** (51 mg, 80 %), by the procedure described for **26**. ^{1}H-NMR ($CDCl_3$): 7.58 and 6.92 (two dt, 2H each, Ar-H), 3.76 and 3.65 (two m, 2H, CH_2OH), 3.54 (m, 2H, $CHCH_2OCH_2$), 3.47 (s, 3H, OCH_3), 3.46-3.41 (m, 3H, $CHCH_2OCH_2$), 2.53 (t, 2H, Ar-CH_2), 1.60-1.50 (m, 4H, Ar-CH_2CH_2 and OCH_2CH_2), 1.35-1.20 (m, 22H, $(CH_2)_{11}$).

1-*O*-[18-(p-Iodophenyl)octadecyl]-2-*O*-methyl-*rac*-glycerol (27)

This compound was synthesized as described for **26** from the benzyl ether **23** (58 mg (0.09 mmol). The alcohol **27** was obtained in a yield of 80 % (40 mg). ^{1}H-NMR ($CDCl_3$): 7.58 and 6.92 (two dt, 2H each,

Ar-H), 3.76 and 3.65 (two m, 2H, CH_2OH), 3.54 (m, 2H, $CHCH_2OCH_2$), 3.47 (s, 3H, OCH_3), 3.46-3.41 (m, 3H, $CHCH_2OCH_2$), 2.53 (t, 2H, Ar-CH_2), 1.60-1.50 (m, 4H, Ar-CH_2CH_2 and OCH_2CH_2), 1.35-1.20 (m, 28H, $(CH_2)_{14}$).

18-(p-Iodophenyl)octadecyl phosphocholine (15)

2-Chloro-2-oxo-1,3,2-dioxaphospholane (0.025 ml; 0.27 mmol) was added to the stirred solution of 18-(p-iodophenyl)octadecanol **13** (115 mg; 0.24 mmol) in benzene (3 ml) containing triethylamine (0.042 ml; 0.29 mmol). Stirring was continued overnight. The precipitated triethylamine hydrochloride was filtered off

and the solvent was removed *in vacuo*. The residue was transferred into a pressure bottle. A solution of trimethylamine in acetonitrile (5 ml; 25 % w/v) was added. The bottle was sealed and heated at 75°C for 24 h. The acetonitrile was then evaporated and the residue was chromatographed on silica gel with chloroform-methanol (gradient from 10:0 to 5:5) followed by final elution with chloroform-methanol-water (65:25:4). After evaporation of the solvent, the product was precipitated by addition of acetone to give a white solid (130 mg; 84 %). ^{1}H-NMR ($CDCl_3$-CD_3OD-D_2O 1:1:0.3): 7.57 and 6.94 (two dt, 2H each, Ar-H), 4.24 (br. m, 2H, $POCH_2CH_2N$), 3.85 (q, 2H, $CH_2POCH_2CH_2N$), 3.61 (m, 2H, CH_2N), 3.21 (s, 9H, $N(CH_3)_3$), 2.55 (2H, t, Ar-CH_2), 1.65-1.55 (m, 4H, Ar-CH_2CH_2 and CH_2CH_2O), 1.35-1.25 (m, 28H, $(CH_2)_{14}$).

15-(p-Iodophenyl)pentadecyl phosphocholine (14)

The introduction of the phosphocholine side chain into **10** (232 mg, 0.54 mmol) was carried out as described for **15**, yielding **14** (231 mg, 72 %) as an amorphous powder.
^{1}H-NMR ($CDCl_3$-CD_3OD-D_2O 1:1:0.3): 7.57 and 6.94 (two dt, 2H each, Ar-H), 4.24 (br. m, 2H, $POCH_2CH_2N$), 3.85 (q, 2H, $CH_2POCH_2CH_2N$), 3.61 (m, 2H, CH_2N), 3.21 (s, 9H, $N(CH_3)_3$), 2.55 (2H, t, Ar-CH_2), 1.65-1.55 (m, 4H, Ar-CH_2CH_2 and CH_2CH_2O), 1.35-1.25 (m, 22H, $(CH_2)_{11}$).

1-*O*-[15-(p-Iodophenyl)pentadecyl]-1,3-propanediol-3-phosphocholine (28)

Alcohol **24** (60 mg, 0.12 mmol) was converted into the phosphocholine **28** (65 mg, 81 %) as described above for **15**. ^{1}H-NMR ($CDCl_3$-CD_3OD-D_2O 1:1:0.3): 7.57 and 6.94 (two dt, 2H each, Ar-H), 4.26 (br. m, 2H, $POCH_2CH_2N$), 3.93 (q, 2H, $CH_2POCH_2CH_2N$), 3.61 (m, 2H, CH_2N), 3.54 (t, 2H, $OCH_2CH_2CH_2OP$), 3.21 (s, 9H, $N(CH_3)_3$), 2.55 (t, 2H, Ar-CH_2), 1.65-1.55 (m, 4H, Ar-CH_2CH_2 and $CH_2CH_2O(CH_2)_3OP$), 1.35-1.25 (m, 28H, $(CH_2)_{14}$).

1-*O*-[15-(p-Iodophenyl)pentadecyl]-2-*O*-methyl-*rac*-glycero-3 phosphocholine (29)

Using the procedure for synthesis of **15**, alcohol **21** (51 mg, 0.1 mmol) was converted to the desired product **29** (55 mg, 82 %). ^{1}H-NMR ($CDCl_3$-CD_3OD-D_2O 1:1:0.3): 7.57 and 6.95 (two dt, 2H each, Ar-H), 4.26 (br. m, 2H, $POCH_2CH_2N$), 3.95 and 3.86 (two m, 2H, $CHCH_2OP$); 3.62 (m, 2H, CH_2N), 3.60-3.50 (m, 3H, $CHCH_2OCH_2$), 3.47 (s, 3H, OCH_3), 3.46 (t, 2H, $CHCH_2OCH_2$), 3.21 (s, 9H, $N(CH_3)_3$), 2.55 (t, 2H, Ar-CH_2), 1.65-1.55 (m, 4H, Ar-CH_2CH_2 and $CH_2CH_2O(CH_2)_3OP$), 1.35-1.22 (m, 22H, m, $(CH_2)_{11}$).

1-*O*-[18-(p-Iodophenyl)octadecyl]-1,3-propanediol-3-phosphocholine (30)

The phosphocholine **30** was synthesized by analogous manner to that of **15** from the alcohol **26** (42 mg; 79 mmol) in 55 % yield (45 mg). ^{1}H-NMR ($CDCl_3$-CD_3OD-D_2O 1:1:0.3): 7.57 and 6.94 (two dt, 2H each, Ar-H), 4.26 (br. m, 2H, $POCH_2CH_2N$), 3.93 (q, 2H, $CH_2POCH_2CH_2N$), 3.61 (m, 2H, CH_2N), 3.54 (t, 2H, $OCH_2CH_2CH_2OP$), 3.21 (s, 9H, $N(CH_3)_3$), 2.55 (t, 2H, Ar-CH_2), 1.65-1.55 (m, 4H, Ar-CH_2CH_2 and $CH_2CH_2O(CH_2)_3OP$), 1.35-1.25 (m, 28H, $(CH_2)_{14}$).

1-*O*-[18-(p-Iodophenyl)octadecyl]-2-*O*-methyl-*rac*-glycero-3-phosphocholine (31)

The phosphocholine **31** was synthesized from the alcohol **27** (33 mg, 0.06 mmol) by the procedure described above for **15** in a yield of 75 % (32 mg). ^{1}H-NMR ($CDCl_3$-CD_3OD-D_2O 1:1:0.3): 7.57 and 6.95 (two dt, 2H each, Ar-H), 4.26 (br. m, 2H, $POCH_2CH_2N$), 3.95 and 3.86 (two m, 2H, $CHCH_2OP$); 3.62 (m, 2H, CH_2N), 3.60-3.50 (m, 3H, $CHCH_2OCH_2$), 3.47 (s, 3H, OCH_3), 3.46 (t, 2H, $CHCH_2OCH_2$), 3.21 (s, 9H, $N(CH_3)_3$), 2.55 (t, 2H, Ar-CH_2), 1.65-1.55 (m, 4H, Ar-CH_2CH_2 and $CH_2CH_2O(CH_2)_3OP$), 1.35-1.22 (m, 28H, m, $(CH_2)_{14}$).

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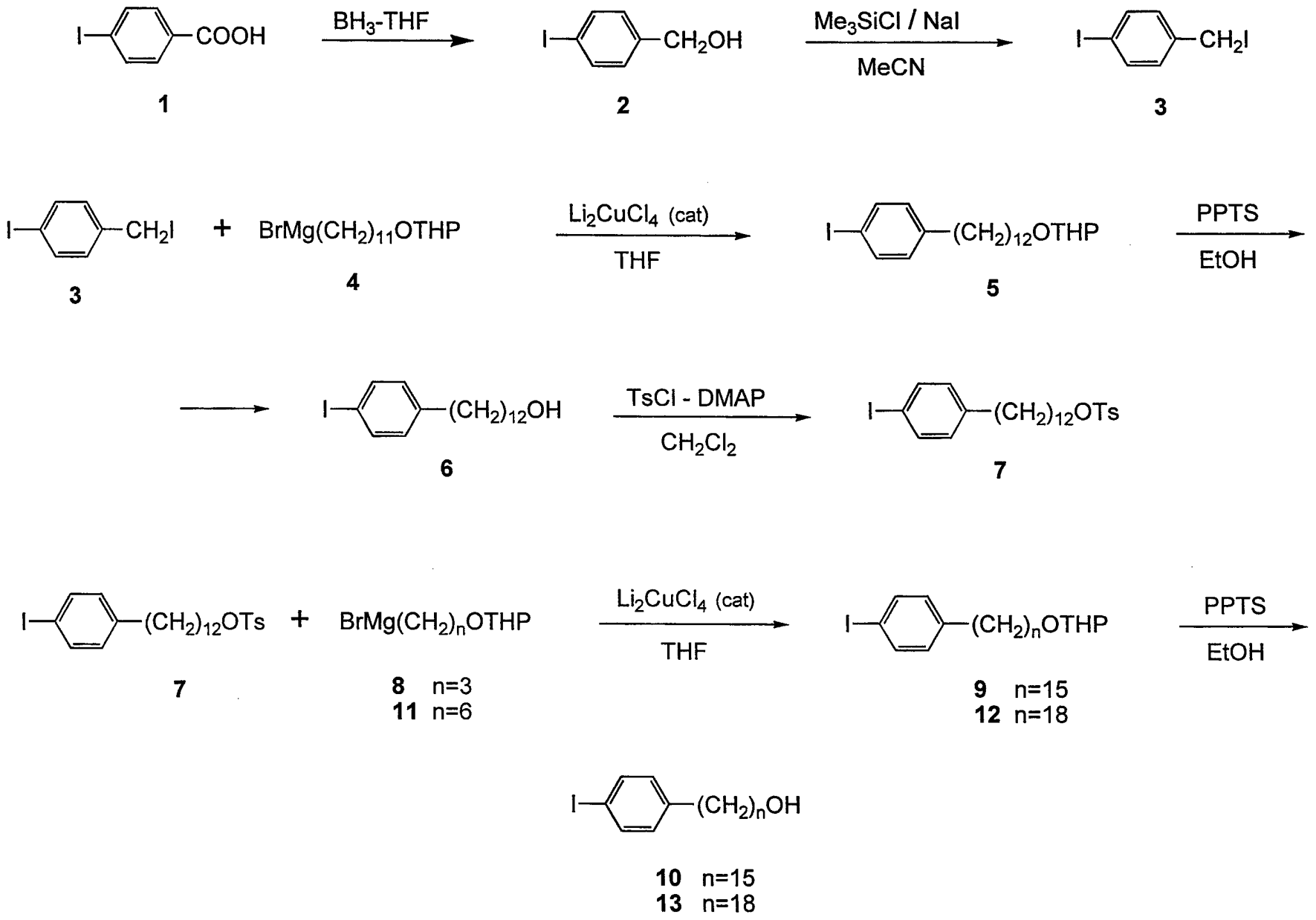
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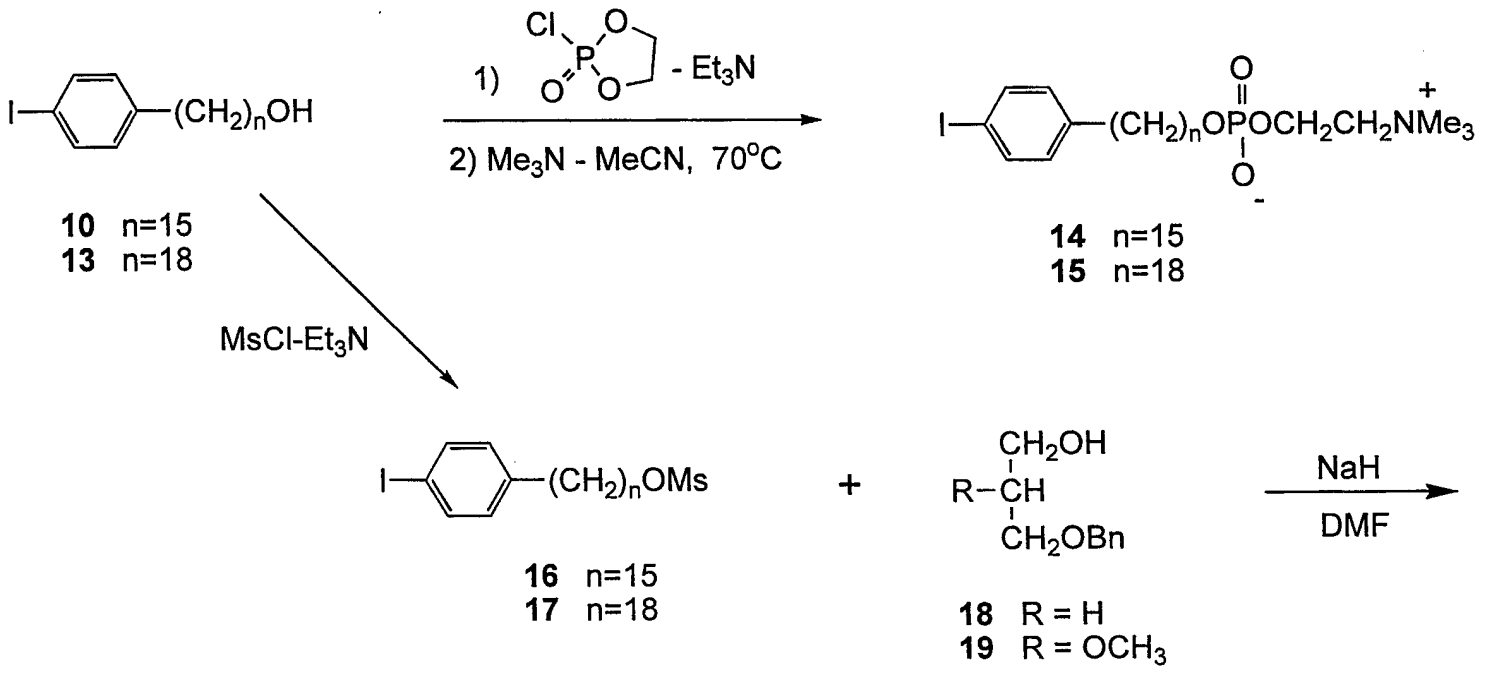
BH3-THF
I—CH2OH
Me3SiCl / NaI
MeCN
I—CH2I
1
2
3
I—CH2I + BrMg(CH2)11OTHP
Li2CuCl4 (cat)
THF
I—(CH2)12OTHP
PPTS
EtOH
4
5
I—(CH2)12OH
TsCl - DMAP
CH2Cl2
I—(CH2)12OTs
6
7
I—(CH2)12OTs + BrMg(CH2)nOTHP
I—(CH2)nOTHP
8 n=3
11 n=6
9 n=15
12 n=18
I—(CH2)nOH
10 n=15
13 n=18



10 n=15
13 n=18

14 n=15
15 n=18

16 n=15
17 n=18

18 R = H
19 R = OCH_3

20 n=15, R = H
21 n=15, R = OCH_3
22 n=18, R = H
23 n=18, R = OCH_3

24 n=15, R = H
25 n=15, R = OCH_3
26 n=18, R = H
27 n=18, R = OCH_3

28 n=15, R = H
29 n=15, R = OCH_3
30 n=18, R = H
31 n=18, R = OCH_3

Appendix 2: Biodistribution of NM-404 in Sprague-Dawley Rats and Dosimetry

NM-404 1hr Biodistribution in Male S-D Rats, n=3.

	dpm/mg ± SEM	% Dose/g ± SEM	% Kg-dose/g ± SEM	% Dose/organ ± SEM
Adrenal	135.385 ± 7.797	0.544 ± 0.029	0.142 ± 0.006	0.036 ± 0.002
Blood	497.461 ±18.618	1.999 ± 0.082	0.525 ± 0.032	25.964 ± 1.575
Bone Marrow	113.891 ±10.203	0.459 ± 0.049	0.120 ± 0.014	0.417 ± 0.047
Duodenum	71.647 ±12.461	0.289 ± 0.055	0.076 ± 0.015	1.462 ± 0.283
Eye	9.075 ± 0.795	0.036 ± 0.003	0.010 ± 0.001	0.013 ± 0.001
Fat	34.658 ± 8.294	0.138 ± 0.031	0.036 ± 0.007	2.544 ± 0.515
Heart	126.931 ± 4.726	0.509 ± 0.012	0.133 ± 0.001	0.385 ± 0.004
Kidney	140.823 ± 5.596	0.565 ± 0.010	0.148 ± 0.002	1.124 ± 0.018
Liver	106.893 ± 14.930	0.427 ± 0.052	0.111 ± 0.011	5.023 ± 0.223
Lung	199.591 ± 5.641	0.803 ± 0.040	0.211 ± 0.014	1.180 ± 0.076
Muscle	25.461 ± 3.846	0.103 ± 0.017	0.027 ± 0.005	12.350 ± 2.273
Plasma	811.593 ±42.200	3.263 ± 0.195	0.857 ± 0.068	23.312 ± 1.856
Prostate	30.705 ± 1.252	0.123 ± 0.004	0.032 ± 0.001	0.000 ± 0.000
Skin	33.565 ± 1.267	0.135 ± 0.004	0.035 ± 0.001	6.349 ± 0.136
Spleen	135.762 ± 6.450	0.546 ± 0.029	0.143 ± 0.007	0.375 ± 0.048
Testes	70.192 ± 3.868	0.282 ± 0.015	0.074 ± 0.005	0.000 ± 0.000
Thyroid	101.968 ±19.269	0.408 ± 0.072	0.106 ± 0.017	0.008 ± 0.001
Bladder	53.677 ± 3.468	0.216 ± 0.019	0.057 ± 0.006	0.000 ± 0.000

NM-404 6hr Biodistribution in Male S-D Rats, n=3.

	dpm/mg ± SEM	% Dose/g ± SEM	% Kg-dose/g ± SEM	% Dose/organ ± SEM
Adrenal	114.576 ±13.659	0.454 ± 0.051	0.110 ± 0.012	0.028 ± 0.003
Blood	231.778 ± 9.400	0.921 ± 0.047	0.224 ± 0.013	11.067 ±0.634
Bone Marrow	83.680 ±11.739	0.332 ± 0.045	0.080 ± 0.011	0.279 ± 0.037
Duodenum	103.310 ± 4.508	0.410 ± 0.019	0.100 ± 0.005	1.921 ± 0.087
Eye	12.729 ± 1.390	0.051 ± 0.005	0.012 ± 0.001	0.016 ± 0.002
Fat	44.221 ± 8.974	0.175 ± 0.034	0.043 ± 0.008	3.012 ± 0.593
Heart	77.057 ± 3.064	0.306 ± 0.013	0.074 ± 0.003	0.215 ± 0.010
Kidney	119.489 ± 9.760	0.474 ± 0.037	0.115 ± 0.008	0.873 ± 0.062
Liver	97.588 ±16.219	0.387 ± 0.063	0.094 ± 0.015	3.946 ± 0.602
Lung	151.461 ±19.696	0.601 ± 0.075	0.146 ± 0.017	0.815 ± 0.096
Muscle	31.809 ± 3.219	0.126 ± 0.012	0.031 ± 0.003	13.914 ± 1.256
Plasma	403.114 ±12.600	1.599 ± 0.038	0.388 ± 0.010	10.557 ± 0.283
Prostate	46.654 ± 5.309	0.185 ± 0.021	0.045 ± 0.005	0.000 ± 0.000
Skin	57.928 ±12.021	0.230 ± 0.048	0.056 ± 0.011	10.043 ± 2.041
Spleen	98.683 ±13.841	0.392 ± 0.054	0.095 ± 0.013	0.286 ± 0.031
Testes	68.325 ± 6.282	0.272 ± 0.029	0.066 ± 0.007	0.000 ± 0.000
Thyroid	89.944 ±17.062	0.356 ± 0.065	0.086 ± 0.015	0.006 ± 0.001
Bladder	86.583 ± 5.787	0.344 ± 0.026	0.084 ± 0.006	0.000 ± 0.000

NM-404 24hr Biodistribution in Male S-D Rats, n=3.

	dpm/mg ± SEM	% Dose/g ± SEM	% Kg-dose/g ± SEM	% Dose/organ ± SEM
Adrenal	160.551 ±11.984	0.640 ± 0.056	0.153 ± 0.014	0.039 ± 0.004
Blood	198.436 ±21.638	0.787 ± 0.077	0.187 ± 0.013	9.245 ± 0.619
Bone Marrow	87.570 ± 5.774	0.349 ± 0.028	0.083 ± 0.007	0.288 ± 0.024
Duodenum	85.915 ±17.702	0.343 ± 0.072	0.081 ± 0.016	1.569 ± 0.304
Eye	19.796 ± 1.254	0.079 ± 0.004	0.019 ± 0.001	0.025 ± 0.002
Fat	36.500 ± 4.767	0.146 ± 0.022	0.035 ± 0.006	2.486 ± 0.446
Heart	67.633 ± 2.141	0.269 ± 0.008	0.064 ± 0.001	0.185 ± 0.004
Kidney	113.747 ± 5.928	0.453 ± 0.032	0.108 ± 0.010	0.824 ± 0.072
Liver	69.516 ± 5.616	0.277 ± 0.027	0.066 ± 0.008	2.876 ± 0.356
Lung	142.638 ± 8.124	0.568 ± 0.041	0.136 ± 0.011	0.761 ± 0.063
Muscle	32.697 ± 2.768	0.130 ± 0.010	0.031 ± 0.001	14.036 ± 0.558
Plasma	339.933 ±39.206	1.348 ± 0.143	0.320 ± 0.023	8.698 ± 0.622
Prostate	76.262 ±12.695	0.304 ± 0.052	0.072 ± 0.011	0.000 ± 0.000
Skin	112.581±10.786	0.446 ± 0.035	0.106 ± 0.007	19.129 ± 1.294
Spleen	93.169 ± 2.529	0.371 ± 0.016	0.089 ± 0.005	0.261 ± 0.051
Testes	73.780 ±10.564	0.293 ± 0.040	0.069 ± 0.007	0.000 ± 0.000
Thyroid	215.059 ±54.844	0.858 ± 0.221	0.203 ± 0.047	0.015 ± 0.004
Bladder	147.558 ±14.536	0.586 ± 0.055	0.139 ± 0.008	0.000 ± 0.000

NM-404 72hr Biodistribution in Male S-D Rats, n=3.

	dpm/mg ± SEM	% Dose/g ± SEM	% Kg-dose/g ± SEM	% Dose/organ ± SEM
Adrenal	130.695 ± 6.602	0.518 ± 0.020	0.136 ± 0.004	0.035 ± 0.001
Blood	185.405 ±11.442	0.734 ± 0.036	0.193 ± 0.003	9.554 ± 0.150
Bone Marrow	84.331 ± 4.550	0.334 ± 0.015	0.088 ± 0.003	0.304 ± 0.009
Duodenum	93.040 ± 7.019	0.368 ± 0.023	0.097 ± 0.003	1.867 ± 0.051
Eye	16.887 ± 1.165	0.067 ± 0.004	0.018 ± 0.000	0.023 ± 0.000
Fat	26.388 ± 3.171	0.104 ± 0.011	0.027 ± 0.002	1.930 ± 0.122
Heart	57.727 ± 2.063	0.229 ± 0.006	0.060 ± 0.002	0.174 ± 0.007
Kidney	92.732 ± 3.917	0.368 ± 0.015	0.097 ± 0.006	0.738 ± 0.049
Liver	50.896 ± 3.300	0.202 ± 0.011	0.053 ± 0.001	2.244 ± 0.137
Lung	126.766 ±11.538	0.502 ± 0.039	0.131 ± 0.004	0.736 ± 0.024
Muscle	26.884 ± 0.294	0.107 ± 0.003	0.028 ± 0.002	12.848 ± 0.959
Plasma	307.746 ±23.657	1.219 ± 0.078	0.320 ± 0.007	8.697 ± 0.188
Prostate	54.520 ± 4.055	0.216 ± 0.014	0.057 ± 0.003	0.000 ± 0.000
Skin	119.139 ± 9.929	0.472 ± 0.033	0.124 ± 0.003	22.256 ± 0.536
Spleen	80.716 ± 6.038	0.320 ± 0.020	0.084 ± 0.001	0.198 ± 0.024
Testes	72.795 ± 4.327	0.288 ± 0.014	0.076 ± 0.003	0.000 ± 0.000
Thyroid	125.261 ±10.922	0.497 ± 0.044	0.131 ± 0.014	0.010 ± 0.001
Bladder	114.843 ±10.522	0.455 ± 0.036	0.119 ± 0.004	0.000 ± 0.000

NM-404 7d Biodistribution in Male S-D Rats, n=3.

	dpm/mg ± SEM	% Dose/g ± SEM	% Kg-dose/g ± SEM	% Dose/organ ± SEM
Adrenal	98.377 ± 2.514	0.429 ± 0.005	0.126 ± 0.002	0.032 ± 0.000
Blood	127.774 ± 2.047	0.557 ± 0.012	0.164 ± 0.007	8.118 ± 0.362
Bone Marrow	64.857 ± 5.236	0.282 ± 0.019	0.083 ± 0.004	0.286 ± 0.013
Duodenum	73.035 ± 4.426	0.318 ± 0.014	0.093 ± 0.002	1.800 ± 0.043
Eye	15.042 ± 0.762	0.066 ± 0.002	0.019 ± 0.001	0.025 ± 0.001
Fat	19.024 ± 1.378	0.083 ± 0.004	0.024 ± 0.001	1.721 ± 0.080
Heart	41.383 ± 1.796	0.180 ± 0.006	0.053 ± 0.002	0.153 ± 0.007
Kidney	73.368 ± 4.521	0.319 ± 0.015	0.094 ± 0.002	0.712 ± 0.018
Liver	41.602 ± 2.542	0.181 ± 0.008	0.053 ± 0.001	2.076 ± 0.042
Lung	90.641 ± 5.044	0.395 ± 0.015	0.116 ± 0.003	0.649 ± 0.018
Muscle	18.565 ± 1.207	0.081 ± 0.004	0.024 ± 0.001	10.795 ± 0.417
Plasma	213.990 ± 4.140	0.934 ± 0.026	0.275 ± 0.014	7.475 ± 0.384
Prostate	42.100 ± 1.466	0.184 ± 0.009	0.054 ± 0.004	0.000 ± 0.000
Skin	84.685 ± 1.628	0.369 ± 0.006	0.109 ± 0.004	19.555 ± 0.757
Spleen	60.015 ± 2.752	0.261 ± 0.007	0.077 ± 0.002	0.164 ± 0.007
Testes	62.066 ± 3.133	0.270 ± 0.009	0.079 ± 0.001	0.000 ± 0.000
Thyroid	162.805 ±15.291	0.708 ± 0.054	0.208 ± 0.014	0.016 ± 0.001
Bladder	83.982 ± 1.284	0.367 ± 0.012	0.108 ± 0.006	0.000 ± 0.000

NM-404 10d Biodistribution in Male S-D Rats, n=3

	dpm/mg ± SEM	% Dose/g ± SEM	% Kg-dsoe/g ± SEM	% Dose/organ ± SEM
Adrenal	88.900 ± 8.207	0.378 ± 0.040	0.116 ± 0.007	0.030 ± 0.002
Blood	114.208 ± 6.498	0.486 ± 0.035	0.150 ± 0.007	7.402 ± 0.340
Bone Marrow	59.916 ± 5.060	0.255 ± 0.024	0.078 ± 0.004	0.271 ± 0.014
Duodenum	66.450 ± 4.183	0.282 ± 0.020	0.087 ± 0.002	1.675 ± 0.048
Eye	13.487 ± 0.774	0.057 ± 0.003	0.018 ± 0.001	0.023 ± 0.001
Fat	24.100 ± 3.002	0.103 ± 0.014	0.032 ± 0.004	2.234 ± 0.264
Heart	35.233 ± 2.163	0.150 ± 0.011	0.046 ± 0.002	0.133 ± 0.005
Kidney	70.284 ± 3.702	0.299 ± 0.020	0.092 ± 0.003	0.699 ± 0.022
Liver	37.548 ± 3.360	0.160 ± 0.016	0.049 ± 0.003	2.062 ± 0.052
Lung	84.354 ± 1.710	0.358 ± 0.009	0.111 ± 0.006	0.621 ± 0.034
Muscle	18.204 ± 1.051	0.077 ± 0.005	0.024 ± 0.002	10.904 ± 0.983
Plasma	188.205 ±10.392	0.800 ± 0.056	0.246 ± 0.009	6.698 ± 0.239
Prostate	39.008 ± 2.255	0.166 ± 0.012	0.051 ± 0.001	0.000 ± 0.000
Skin	83.979 ± 3.314	0.357 ± 0.017	0.110 ± 0.001	19.782 ± 0.143
Spleen	53.956 ± 5.370	0.230 ± 0.025	0.070 ± 0.005	0.173 ± 0.007
Testes	61.869 ± 3.959	0.263 ± 0.018	0.081 ± 0.003	0.000 ± 0.000
Thyroid	131.373 ±13.766	0.557 ± 0.055	0.172 ± 0.016	0.013 ± 0.001
Bladder	80.858 ± 5.609	0.344 ± 0.028	0.106 ± 0.007	0.000 ± 0.000

MIRDOSE (IBM PC VERSION 3.1 - AUGUST 1995)

Radiation Dose Estimates for the REFERENCE ADULT
for 131-I-53 NM-404

	TARGET ORGAN	TOTAL DOSE mGy/MBq	TOTAL DOSE rad/mCi	PRIMARY CONTRIBUTOR	%	SECONDARY CONTRIBUTOR	%
1)	Adrenals	6.14E-01	2.27E+00	Adrenals	83.7%	Muscle	8.7%
2)	Brain	1.54E-02	5.71E-02	Muscle	74.0%	Red Marrow	20.6%
3)	Breasts	3.94E-02	1.46E-01	Muscle	55.7%	Lungs	25.5%
4)	Gallbladder Wall	9.92E-02	3.67E-01	Muscle	55.1%	Liver	31.9%
5)	LLI Wall	7.01E-02	2.59E-01	Muscle	86.9%	Red Marrow	10.1%
6)	Small Intestine	6.55E-02	2.43E-01	Muscle	77.9%	Red Marrow	9.7%
7)	Stomach	7.17E-02	2.65E-01	Muscle	70.2%	Liver	8.0%
8)	ULI Wall	6.63E-02	2.45E-01	Muscle	75.5%	Liver	10.5%
9)	Heart Wall	2.98E-01	1.10E+00	Heart Wall	75.0%	Muscle	14.2%
10)	Kidneys	4.99E-01	1.85E+00	Kidneys	85.8%	Muscle	9.3%
11)	Liver	3.41E-01	1.26E+00	Liver	84.8%	Muscle	10.4%
12)	Lungs	5.61E-01	2.08E+00	Lungs	89.5%	Muscle	7.6%
13)	Muscle	2.39E-01	8.86E-01	Muscle	94.6%	Lungs	1.7%
14)	Ovaries	7.59E-02	2.81E-01	Muscle	86.2%	Red Marrow	9.7%
15)	Pancreas	9.72E-02	3.60E-01	Muscle	58.3%	Liver	14.2%
16)	Red Marrow	2.40E-01	8.89E-01	Red Marrow	76.6%	Muscle	18.4%
17)	Bone Surfaces	1.65E-01	6.12E-01	Red Marrow	62.3%	Muscle	31.8%
18)	Skin	3.69E-02	1.37E-01	Muscle	82.4%	Lungs	5.2%
19)	Spleen	4.10E-01	1.52E+00	Spleen	83.0%	Muscle	11.8%
20)	Testes	3.86E-01	1.43E+00	Testes	87.0%	Muscle	12.6%
21)	Thymus	7.21E-02	2.67E-01	Muscle	69.3%	Lungs	16.4%
22)	Thyroid	8.23E-01	3.04E+00	Thyroid	92.5%	Muscle	6.6%
23)	Urin Bladder Wall	6.86E-02	2.54E-01	Muscle	93.1%	Red Marrow	4.6%
24)	Uterus	7.28E-02	2.69E-01	Muscle	89.1%	Red Marrow	7.2%
27)	Total Body	1.51E-01	5.57E-01	Muscle	75.6%	Lungs	7.1%
28)	EFF DOSE EQUIV	3.58E-01	1.32E+00	Remainder	36.2%	Gonads	26.9%
29)	EFF DOSE	2.83E-01	1.05E+00	Gonads	27.2%	Lungs	23.8%

Units of EDE and ED are mSv/MBq or rem/mCi.

RESIDENCE TIMES:

Adrenals	7.30E-02 hr
Heart Wall	5.75E-01 hr
Kidneys	1.02E+00 hr
Liver	3.78E+00 hr
Lungs	4.17E+00 hr
Muscle	4.50E+01 hr
Red Marrow	3.43E+00 hr
Spleen	4.90E-01 hr
Testes	1.10E-01 hr
Thyroid	1.35E-01 hr

MIRDOSE 3.1 Source Files:

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MASTER3.DAT	56440	3/30/94 10:30:00 AM
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ONSKEL	419776	10/28/94 1:56:08 AM
NBSKEL	419776	10/28/94 2:42:52 AM

Radiation Dose Estimates for the REFERENCE ADULT for I131 NM-404

Target Organ	rad / mCi	rad / 2mCi total dose
Adrenals	2.27	4.54
Brain	0.057	0.114
Breasts	0.146	0.292
Gallbladder Wall	0.367	0.734
LLI Wall	0.259	0.518
Small Intestine	0.243	0.486
Stomach	0.265	0.530
ULI Wall	0.245	0.490
Heart Wall	1.10	2.20
Kidneys	1.85	3.70
Liver	1.26	2.52
Lungs	2.08	4.16
Muscle	0.886	1.772
Ovaries	0.281	0.562
Pancreas	0.360	0.72
Red Marrow	0.889	1.778
Bone Surfaces	0.612	1.224
Skin	0.137	0.274
Spleen	1.52	3.04
Testes	1.43	2.86
Thymus	0.267	0.534
Thyroid	3.04	6.08
Urine Bladder Wall	0.254	0.508
Uterus	0.269	0.538
Total Body	0.557	1.114

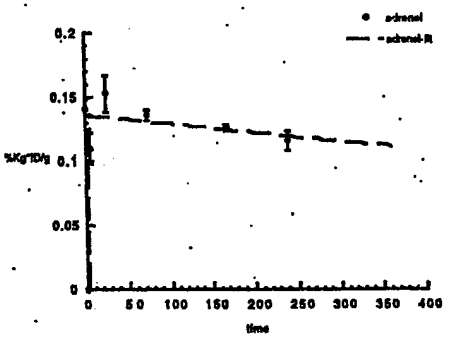
N	time	%Kg*ID/g adrenal	error	blood	error	marrow	error	duodenum	error	eye	error
	0.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	1.0	0.142	0.006	0.525	0.032	0.120	0.014	0.076	0.015	0.010	0.001
3	6.0	0.110	0.012	0.224	0.013	0.080	0.011	0.100	0.005	0.012	0.001
3	24.0	0.153	0.014	0.187	0.013	0.083	0.007	0.081	0.016	0.019	0.001
3	72.0	0.136	0.004	0.193	0.003	0.088	0.003	0.097	0.003	0.018	0.000
3	168.0	0.126	0.002	0.164	0.007	0.083	0.004	0.093	0.002	0.019	0.001
3	240.0	0.116	0.007	0.150	0.007	0.078	0.004	0.087	0.002	0.018	0.001

		T1/2	
Extrapolation to reference man:	73.7 Kg	I-131:	193.4 h

0.003584 /h

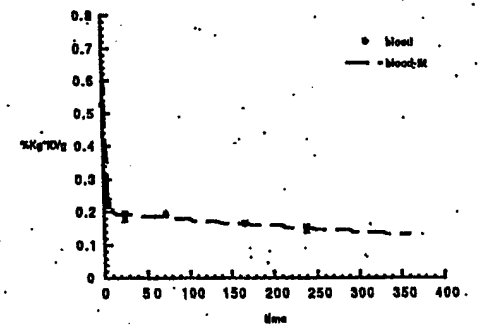
adrenal

Goodness-of-fit stat		ref man %ID/g-h:	0.4487		
		organ mass:	16.3 g		
Weighted sum of squared obs:	0.1035	%ID-h:	7.313		
Sum of squared deviations:	0.0011	residence time:	0.073 h		
Standard deviation of data:	0.0190				
r-squared:	0.9895				
Coeff of determination:	0.9319				
Correlation:	0.9653				
Model Selection Criterion:	1.5437				
Weighting Factor:	0.0000				
computation time:	0.94 secs	A[1]=	0.13652	k[1]=	0.000543
calculated lag time:	0.0000	A[2]=	-0.13642	k[2]=	10.341
sum of squared residuals:	0.0011				
Model Selection Criterion:	1.5437				
Weighting Factor:	0.0000				



blood

Goodness-of-fit statisti		ref man %ID/g-h:	0.5880		
		organ mass:	5400 g		
Weighted sum of squared obs:	0.4474	%ID-h:	3175.452		
Sum of squared deviations:	0.0001	residence time:	31.755 h		
Standard deviation of data:	0.0083				
r-squared:	0.9997	Dose:	1.874 rad/mCi		
Coeff of determination:	0.9986	ref: table 8, p334, Cloutier and Watson			
Correlation:	0.9993				
Model Selection Criterion:	5.2486				
Weighting Factor:	0.0000				
computation time:	0.38 secs	A[1]=	0.19903	k[1]=	0.001124
calculated lag time:	0.0000	A[2]=	0.53971	k[2]=	0.50354
sum of squared residuals:	0.0001				
Model Selection Criterion:	5.2486				
Weighting Factor:	0.0000				

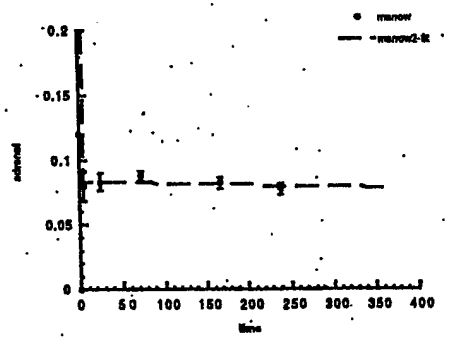


fat		error	heart	error	kidney	error	liver	error	lung	error	muscle	error	plasma	error	prostate	error	skin	error
	0.000	0.0001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0.036	0.0070	0.133	0.001	0.148	0.002	0.111	0.011	0.211	0.014	0.027	0.005	0.857	0.068	0.032	0.001	0.035	0.001
	0.043	0.0080	0.074	0.003	0.115	0.008	0.094	0.015	0.146	0.017	0.031	0.003	0.388	0.010	0.045	0.005	0.056	0.011
	0.035	0.0060	0.064	0.001	0.108	0.010	0.086	0.008	0.136	0.011	0.031	0.001	0.320	0.023	0.072	0.011	0.106	0.007
	0.027	0.0020	0.060	0.002	0.097	0.006	0.053	0.001	0.131	0.004	0.028	0.002	0.320	0.007	0.057	0.003	0.124	0.003
	0.024	0.0010	0.053	0.002	0.094	0.002	0.053	0.001	0.116	0.003	0.024	0.001	0.275	0.014	0.054	0.004	0.109	0.004
	0.032	0.0040	0.046	0.002	0.092	0.003	0.049	0.003	0.111	0.006	0.024	0.002	0.246	0.009	0.051	0.001	0.110	0.001

spleen	error	testes	error	thyroid	error	bladder	error
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.143	0.007	0.074	0.005	0.106	0.017	0.057	0.006
0.095	0.013	0.066	0.007	0.086	0.015	0.084	0.006
0.089	0.005	0.069	0.007	0.203	0.047	0.139	0.008
0.084	0.001	0.076	0.003	0.131	0.014	0.119	0.004
0.077	0.002	0.079	0.001	0.208	0.014	0.108	0.006
0.070	0.005	0.081	0.003	0.172	0.016	0.106	0.007

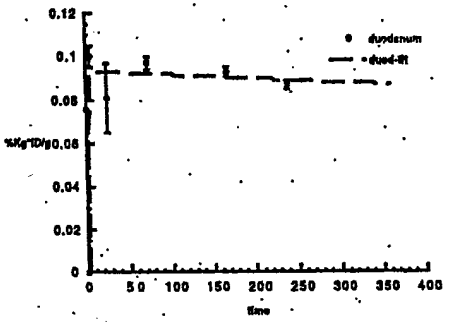
marrow

Goodness-of-fit statistl	cs:		ref man %ID/g-h:	0.3064	
			organ mass:	1120	g
Weighted sum of squared obs:	0.0484		%ID-h:	343.114	
Sum of squared deviations:	0.0000		residence time:	3.431	h
Standard deviation of data:	0.0050				
r-squared:	0.9990				
Coeff of determination:	0.9598				
Correlation:	0.9797				
Model Selection Criterion:	1.8803				
Weighting Factor:	0.0000				
computation time:	1.15 secs	A[1]=	0.083796	k[1]=	0.000166
calculated lag time:	0.0000	A[2]=	0.6771	k[2]=	2.9283
sum of squared residuals:	0.0000				
Model Selection Criterion:	1.8803				
Weighting Factor:	0.0000				



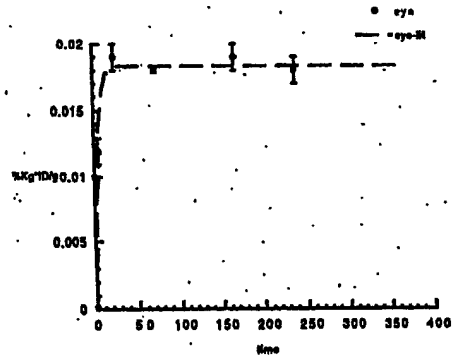
duodenum

Goodness-of-fit statistl	cs:		ref man %ID/g-h:	0.3351	
			organ mass:		g
Weighted sum of squared obs:	0.0480		%ID-h:	0.000	
Sum of squared deviations:	0.0002		residence time:	0.000	h
Standard deviation of data:	0.0086				
r-squared:	0.9953				
Coeff of determination:	0.9690				
Correlation:	0.9844				
Model Selection Criterion:	2.3310				
Weighting Factor:	0.0000				
computation time:	0.44 secs	A[1]=	0.093353	k[1]=	0.000187
calculated lag time:	0.0000	A[2]=	-0.09326	k[2]=	1.6837
sum of squared residuals:	0.0002				
Model Selection Criterion:	2.3310				
Weighting Factor:	0.0000				



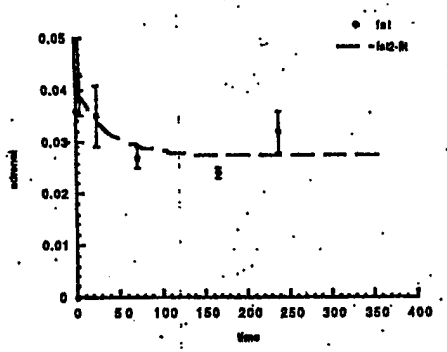
eye

Goodness-of-fit statistl	cs:		ref man %ID/g-h:	0.0683	
			organ mass:		g
Weighted sum of squared obs:	0.0016		%ID-h:	0.000	
Sum of squared deviations:	0.0000		residence time:	0.000	h
Standard deviation of data:	0.0032				
r-squared:	0.9812				
Coeff of determination:	0.8970				
Correlation:	0.9471				
Model Selection Criterion:	1.1300				
Weighting Factor:	0.0000				
computation time:	0.27 secs	A[1]=	0.018388	k[1]=	1.73E-05
calculated lag time:	0.0000	A[2]=	-0.01551	k[2]=	0.21305
sum of squared residuals:	0.0000				
Model Selection Criterion:	1.1300				
Weighting Factor:	0.0000				



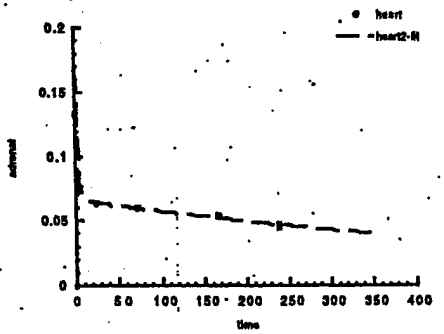
fat

Goodness-of-fit statist	lcs:		ref man %ID/g-h:	0.1095	
			organ mass:		g
Weighted sum of squared obs:		0.0067	%ID-h:	0.000	
Sum of squared deviations:		0.0001	residence time:	0.000	h
Standard deviation of data:		0.0062			
r-squared:		0.9886			
Coeff of determination:		0.6687			
Correlation:		0.8177			
Model Selection Criterion:		-0.2287			
Weighting Factor:		0.0000			
computation time:	0.00	secs	A[1]=	0.027448	k[1]= 7.32E-06
calculated lag time:		0.0000	A[2]=	0.01291	k[2]= 0.026736
sum of squared residuals:		0.0001			
Model Selection Criterion:		-0.2287			
Weighting Factor:		0.0000			



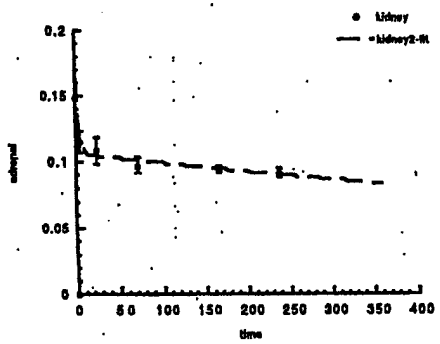
heart

Goodness-of-fit statisti	cs:		ref man %ID/g-h:	0.1819	
			organ mass:	316	g
Weighted sum of squared obs:		0.0358	%ID-h:	57.485	
Sum of squared deviations:		0.0000	residence time:	0.575	h
Standard deviation of data:		0.0009			
r-squared:		1.0000			
Coeff of determination:		0.9997			
Correlation:		0.9998			
Model Selection Criterion:		6.6577			
Weighting Factor:		0.0000			
computation time:	0.39	secs	A[1]=	0.066642	k[1]= 0.001476
calculated lag time:		0.0000	A[2]=	0.10163	k[2]= 0.42479
sum of squared residuals:		0.0000			
Model Selection Criterion:		6.6577			
Weighting Factor:		0.0000			



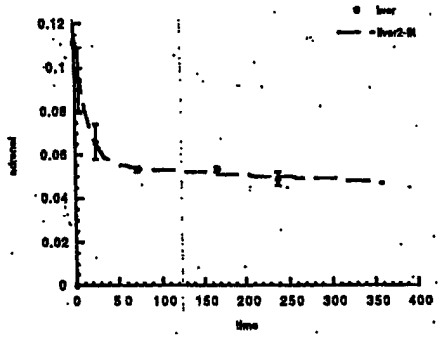
kidney

Goodness-of-fit statisti	cs:		ref man %ID/g-h:	0.3410	
			organ mass:	299	g
Weighted sum of squared obs:		0.0735	%ID-h:	101.973	
Sum of squared deviations:		0.0000	residence time:	1.020	h
Standard deviation of data:		0.0041			
r-squared:		0.9996			
Coeff of determination:		0.9852			
Correlation:		0.9926			
Model Selection Criterion:		2.8788			
Weighting Factor:		0.0000			
computation time:	0.27	secs	A[1]=	0.10619	k[1]= 0.000672
calculated lag time:		0.0000	A[2]=	0.056552	k[2]= 0.30067
sum of squared residuals:		0.0000			
Model Selection Criterion:		2.8788			
Weighting Factor:		0.0000			



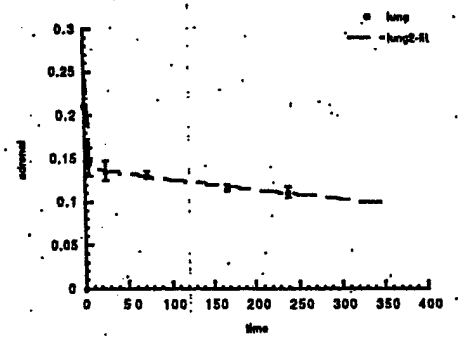
liver

Goodness-of-fit statisti	cs:		ref man %ID/g-h:	0.1981	
			organ mass:	1910	g
Weighted sum of squared obs:	0.0335		%ID-h:	378.338	
Sum of squared deviations:	0.0000		residence time:	3.783	h
Standard deviation of data:	0.0015				
r-squared:	0.9999				
Coeff of determination:	0.9987				
Correlation:	0.9994				
Model Selection Criterion:	5.3077				
Weighting Factor:	0.0000				
computation time:	0.66 secs	A[1]=	0.055087	k[1]=	0.000412
calculated lag time:	0.0000	A[2]=	0.059843	k[2]=	0.069991
sum of squared residuals:	0.0000				
Model Selection Criterion:	5.3077				
Weighting Factor:	0.0000				



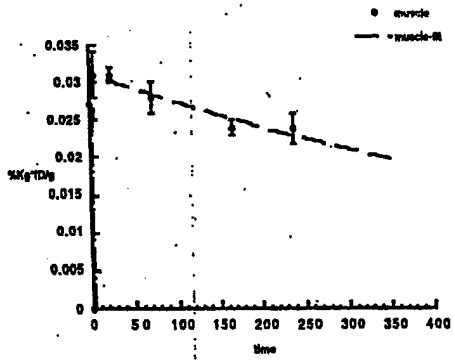
lung

Goodness-of-fit statisti	cs:		ref man %ID/g-h:	0.4167	
			organ mass:	1000	g
Weighted sum of squared obs:	0.1273		%ID-h:	416.652	
Sum of squared deviations:	0.0000		residence time:	4.167	h
Standard deviation of data:	0.0018				
r-squared:	1.0000				
Coeff of determination:	0.9990				
Correlation:	0.9995				
Model Selection Criterion:	5.5489				
Weighting Factor:	0.0000				
computation time:	0.38 secs	A[1]=	0.13955	k[1]=	0.000997
calculated lag time:	0.0000	A[2]=	0.11307	k[2]=	0.457
sum of squared residuals:	0.0000				
Model Selection Criterion:	5.5489				
Weighting Factor:	0.0000				



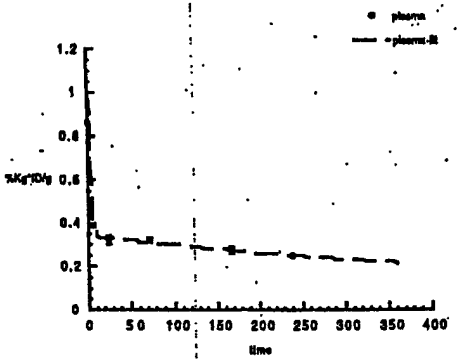
muscle

Goodness-of-fit statisti	cs:		ref man %ID/g-h:	0.0870	
			organ mass:	51800	g
Weighted sum of squared obs:	0.0046		%ID-h:	4504.819	
Sum of squared deviations:	0.0000		residence time:	45.048	h
Standard deviation of data:	0.0010				
r-squared:	0.9993				
Coeff of determination:	0.9954				
Correlation:	0.9977				
Model Selection Criterion:	4.2313				
Weighting Factor:	0.0000				
computation time:	0.38 secs	A[1]=	0.031243	k[1]=	0.001279
calculated lag time:	0.0000	A[2]=	-0.03114	k[2]=	2.0027
sum of squared residuals:	0.0000				
Model Selection Criterion:	4.2313				
Weighting Factor:	0.0000				



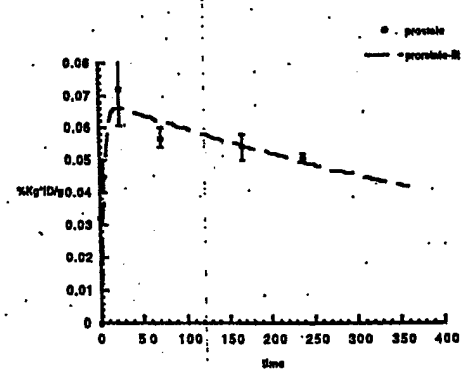
plasma

Goodness-of-fit statistl	cs:		ref man %ID/g-h:	0.9715	
			organ mass:	g	
Weighted sum of squared obs:		1.2259	%ID-h:	0.000	
Sum of squared deviations:		0.0002	residence time:	0.000	h
Standard deviation of data:		0.0102			
r-squared:		0.9998			
Coeff of determination:		0.9992			
Correlation:		0.9996			
Model Selection Criterion:		5.8050			
Weighting Factor:		0.0000			
computation time:	0.38	secs	A[1]=	0.33848	k[1]= 0.001264
calculated lag time:		0.0000	A[2]=	0.82193	k[2]= 0.45984
sum of squared residuals:		0.0002			
Model Selection Criterion:		5.8050			
Weighting Factor:		0.0000			



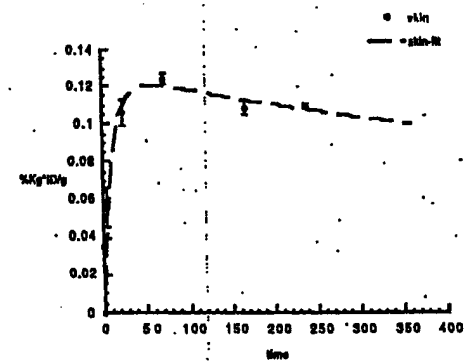
prostate

Goodness-of-fit statistl	cs:		ref man %ID/g-h:	0.1839	
			organ mass:	g	
Weighted sum of squared obs:		0.0170	%ID-h:	0.000	
Sum of squared deviations:		0.0003	residence time:	0.000	h
Standard deviation of data:		0.0103			
r-squared:		0.9811			
Coeff of determination:		0.8988			
Correlation:		0.9481			
Model Selection Criterion:		1.1479			
Weighting Factor:		0.0000			
computation time:	0.38	secs	A[1]=	0.068615	k[1]= 0.001375
calculated lag time:		0.0000	A[2]=	-0.06023	k[2]= 0.21039
sum of squared residuals:		0.0003			
Model Selection Criterion:		1.1479			
Weighting Factor:		0.0000			



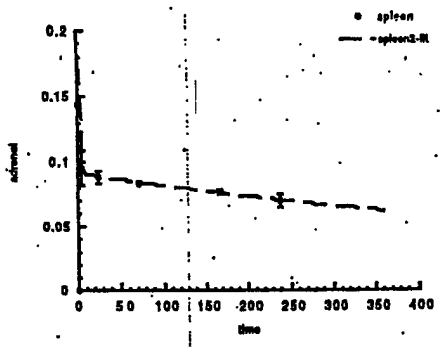
skin

Goodness-of-fit statistl	cs:		ref man %ID/g-h:	0.3869	
			organ mass:	g	
Weighted sum of squared obs:		0.0550	%ID-h:	0.000	
Sum of squared deviations:		0.0004	residence time:	0.000	h
Standard deviation of data:		0.0113			
r-squared:		0.9931			
Coeff of determination:		0.9714			
Correlation:		0.9856			
Model Selection Criterion:		2.4112			
Weighting Factor:		0.0000			
computation time:	0.44	secs	A[1]=	0.126	k[1]= 0.000643
calculated lag time:		0.0000	A[2]=	-0.11404	k[2]= 0.084513
sum of squared residuals:		0.0004			
Model Selection Criterion:		2.4112			
Weighting Factor:		0.0000			



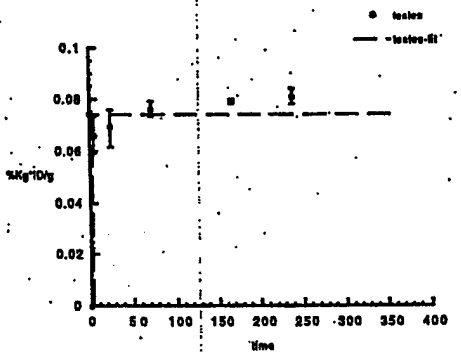
spleen

Goodness-of-fit statisti	cs:		ref man %ID/g-h:	0.2680	
			organ mass:	183 g	
Weighted sum of squared obs:	0.0553		%ID-h:	49.048	
Sum of squared deviations:	0.0000		residence time:	0.490 h	
Standard deviation of data:	0.0008				
r-squared:	1.0000				
Coeff of determination:	0.9997				
Correlation:	0.9998				
Model Selection Criterion:	6.6592				
Weighting Factor:	0.0000				
computation time:	0.38 secs	A[1]=	0.091237	k[1]=	0.001075
calculated lag time:	0.0000	A[2]=	0.085137	k[2]=	0.49571
sum of squared residuals:	0.0000				
Model Selection Criterion:	6.6592				
Weighting Factor:	0.0000				



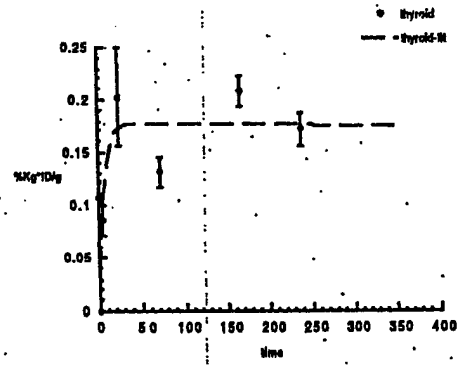
testes

Goodness-of-fit statistics	:		ref man %ID/g-h:	0.2807	
			organ mass:	39.1 g	
Weighted sum of squared obs:	0.0332		%ID-h:	10.977	
Sum of squared deviations:	0.0002		residence time:	0.110 h	
Standard deviation of data:	0.0075				
r-squared:	0.9950				
Coeff of determination:	0.9657				
Correlation:	0.9827				
Model Selection Criterion:	2.2310				
Weighting Factor:	0.0000				
computation time:	1.21 secs	A[1]=	0.0742	k[1]=	1.51E-12
calculated lag time:	0.0000	A[2]=	-0.0741	k[2]=	5.9149
sum of squared residuals:	0.0002				
Model Selection Criterion:	2.2310				
Weighting Factor:	0.0000				



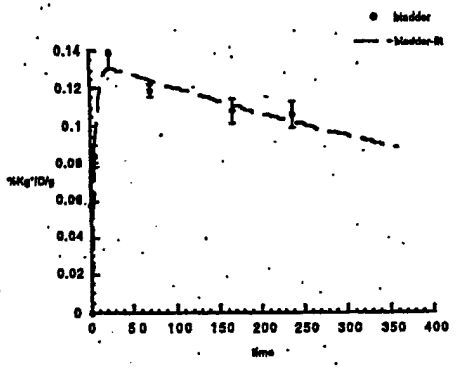
thyroid

Goodness-of-fit statistic	s:		ref man %ID/g-h:	0.6515	
			organ mass:	20.7 g	
Weighted sum of squared obs:	0.1499		%ID-h:	13.486	
Sum of squared deviations:	0.0089		residence time:	0.135 h	
Standard deviation of data:	0.0546				
r-squared:	0.9404				
Coeff of determination:	0.7257				
Correlation:	0.8519				
Model Selection Criterion:	0.1506				
Weighting Factor:	0.0000				
computation time:	0.55 secs	A[1]=	0.17879	k[1]=	5.98E-05
calculated lag time:	0.0000	A[2]=	-0.14366	k[2]=	0.13291
sum of squared residuals:	0.0089				
Model Selection Criterion:	0.1506				
Weighting Factor:	0.0000				



bladder

Goodness-of-fit statistics	:		ref man %ID/g-h:	0.3776	
			organ mass:	47.6	g
Weighted sum of squared obs:	0.0667		%ID-h:	17.972	
Sum of squared deviations:	0.0009		residence time:	0.180	h
Standard deviation of data:	0.0178				
r-squared:	0.9858				
Coeff of determination:	0.9272				
Correlation:	0.9629				
Model Selection Criterion:	1.4772				
Weighting Factor:	0.0000				
computation time:	0.39 secs	A[1]=	0.13585	k[1]=	0.001186
calculated lag time:	0.0000	A[2]=	-0.12071	k[2]=	0.18113
sum of squared residuals:	0.0009				
Model Selection Criterion:	1.4772				
Weighting Factor:	0.0000				



Appendix 3: Biodistribution of NM-412 in Sprague-Dawley Rats and Dosimetry

TABLE 1. BIODISTRIBUTION OF ^{125}I-NM-412 IN 2% TWEEN 20/STERILE WATER IN MALE SPRAGUE-DAWLEY RATS FOLLOWING I.V. INJECTION.

NM-412 1 Day (n=4)	dpm/mg	% dose/gm ± SEM	% kg-does/gm ± SEM	% dose/organ ± SEM
Adrenal	122.649 ±9.922	0.939 ± 0.092	0.219 ± 0.020	0.056 ± 0.005
Blood	30.400 ±1.536	0.233 ± 0.017	0.054 ± 0.003	2.689 ± 0.169
Bone Marrow	50.994 ± 3.887	0.390 ± 0.036	0.091 ± 0.008	0.316 ± 0.027
Duodenum	46.064 ± 4.552	0.354 ± 0.044	0.082 ± 0.009	1.591 ± 0.174
Eye	5.468 ± 0.887	0.042 ± 0.008	0.010 ± 0.002	0.013 ± 0.002
Fat	18.999 ± 2.646	0.145 ± 0.021	0.034 ± 0.005	2.410 ± 0.360
Heart	21.540 ± 1.503	0.164 ± 0.012	0.038 ± 0.003	0.111 ± 0.008
Kidney	57.235 ± 3.774	0.438 ± 0.036	0.102 ± 0.008	0.778 ± 0.059
Liver	49.792 ± 2.917	0.380 ± 0.022	0.089 ± 0.006	3.880 ± 0.105
Lung	63.950 ± 7.836	0.487 ± 0.058	0.114 ± 0.014	0.639 ± 0.078
Muscle	10.650 ± 0.876	0.081 ± 0.008	0.019 ± 0.002	8.641 ± 0.697
Plasma	42.631 ± 2.541	0.326 ± 0.027	0.076 ± 0.005	2.073 ± 0.149
Prostate	20.251 ± 1.490	0.155 ± 0.014	0.036 ± 0.003	0.000 ± 0.000
Skin	27.142 ± 2.986	0.207 ± 0.023	0.049 ± 0.006	8.733 ± 1.008
Spleen	55.069 ± 2.844	0.420 ± 0.025	0.098 ± 0.006	0.293 ± 0.021
Testes	15.503 ± 1.356	0.119 ± 0.012	0.028 ± 0.003	0.000 ± 0.000
Thyroid	4624.799 ± 218.561	35.321 ± 2.015	8.262 ± 0.479	0.620 ± 0.036
Urinary Bladder	31.393 ± 3.050	0.241 ± 0.028	0.056 ± 0.006	0.000 ± 0.000
NM-412 3 Day (n=4)				
Adrenal	89.169 ±5.668	0.687 ± 0.030	0.172 ± 0.005	0.044 ± 0.001
Blood	12.081 ± 0.396	0.094 ± 0.004	0.023 ± 0.001	1.160 ± 0.063
Bone Marrow	31.589 ± 1.256	0.244 ± 0.011	0.061 ± 0.003	0.212 ± 0.009
Duodenum	22.852 ± 1.413	0.176 ± 0.009	0.044 ± 0.002	0.852 ± 0.048
Eye	4.429 ± 0.227	0.034 ± 0.001	0.009 ± 0.000	0.011 ± 0.001
Fat	20.378 ± 2.393	0.157 ± 0.018	0.039 ± 0.005	2.795 ± 0.349
Heart	9.448 ± 0.398	0.073 ± 0.004	0.018 ± 0.001	0.053 ± 0.003
Kidney	32.284 ± 0.844	0.249 ± 0.004	0.062 ± 0.002	0.474 ± 0.012
Liver	23.407 ± 0.637	0.181 ± 0.007	0.045 ± 0.002	1.843 ± 0.058
Lung	34.258 ± 0.999	0.265 ± 0.013	0.066 ± 0.003	0.372 ± 0.019
Muscle	5.213 ± 0.145	0.040 ± 0.002	0.010 ± 0.001	4.604 ± 0.257
Plasma	17.059 ± 0.787	0.132 ± 0.007	0.033 ± 0.002	0.899 ± 0.052
Prostate	13.141 ± 1.110	0.101 ± 0.007	0.025 ± 0.002	0.000 ± 0.000
Skin	21.706 ± 1.855	0.168 ± 0.015	0.042 ± 0.003	7.544 ± 0.621
Spleen	29.845 ± 1.874	0.230 ± 0.013	0.058 ± 0.003	0.127 ± 0.005
Testes	12.930 ± 0.385	0.100 ± 0.002	0.025 ± 0.000	0.000 ± 0.000
Thyroid	2896.927 ± 90.447	22.371 ± 0.307	5.598 ± 0.123	0.420 ± 0.009
Urinary Bladder	22.101 ± 1.349	0.170 ± 0.007	0.043 ± 0.002	0.000 ± 0.000

NM-412 5 Day (n=4)	dpm/mg	% dose/gm ± SEM	% kg-does/gm ± SEM	% dose/organ ± SEM
Adrenal	61.839 ± 5.512	0.446 ± 0.033	0.089 ± 0.006	0.023 ± 0.002
Blood	7.224 ± 1.305	0.052 ± 0.008	0.010 ± 0.002	0.511 ± 0.083
Bone Marrow	20.064 ± 1.238	0.145 ± 0.008	0.029 ± 0.001	0.100 ± 0.004
Duodenum	16.391 ± 1.940	0.118 ± 0.013	0.023 ± 0.002	0.453 ± 0.045
Eye	4.448 ± 0.625	0.032 ± 0.004	0.006 ± 0.001	0.008 ± 0.001
Fat	25.814 ± 1.983	0.187 ± 0.016	0.037 ± 0.003	2.644 ± 0.221
Heart	6.462 ± 0.443	0.047 ± 0.002	0.009 ± 0.000	0.027 ± 0.001
Kidney	25.904 ± 1.535	0.187 ± 0.008	0.037 ± 0.001	0.283 ± 0.010
Liver	22.883 ± 1.663	0.165 ± 0.009	0.033 ± 0.002	1.002 ± 0.059
Lung	24.734 ± 3.568	0.177 ± 0.022	0.035 ± 0.004	0.198 ± 0.025
Muscle	4.967 ± 0.976	0.036 ± 0.008	0.007 ± 0.002	3.308 ± 0.747
Plasma	8.581 ± 2.476	0.061 ± 0.016	0.012 ± 0.003	0.330 ± 0.091
Prostate	9.205 ± 0.897	0.067 ± 0.007	0.013 ± 0.001	0.000 ± 0.000
Skin	20.517 ± 0.638	0.148 ± 0.004	0.030 ± 0.001	5.335 ± 0.208
Spleen	22.204 ± 1.707	0.161 ± 0.012	0.032 ± 0.002	0.072 ± 0.003
Testes	12.848 ± 0.689	0.093 ± 0.003	0.018 ± 0.001	0.000 ± 0.000
Thyroid	3523.361 ± 240.666	25.537 ± 1.951	5.078 ± 0.315	0.381 ± 0.024
Urinary Bladder	15.425 ± 1.817	0.111 ± 0.011	0.022 ± 0.002	0.000 ± 0.000
NM-412 8 Day (n=4)				
Adrenal	29.256 ± 1.685	0.259 ± 0.017	0.070 ± 0.003	0.018 ± 0.001
Blood	1.798 ± 0.108	0.016 ± 0.001	0.004 ± 0.000	0.215 ± 0.014
Bone Marrow	6.721 ± 0.963	0.059 ± 0.009	0.016 ± 0.002	0.056 ± 0.007
Duodenum	4.901 ± 0.284	0.043 ± 0.002	0.004 ± 0.001	0.006 ± 0.001
Fat	22.953 ± 1.155	0.203 ± 0.009	0.055 ± 0.003	3.925 ± 0.244
Heart	1.943 ± 0.144	0.017 ± 0.001	0.005 ± 0.000	0.013 ± 0.001
Kidney	7.156 ± 0.619	0.063 ± 0.006	0.017 ± 0.001	0.130 ± 0.009
Liver	6.457 ± 0.207	0.057 ± 0.002	0.016 ± 0.001	0.588 ± 0.019
Lung	7.155 ± 0.909	0.063 ± 0.009	0.017 ± 0.002	0.096 ± 0.011
Muscle	1.201 ± 0.160	0.011 ± 0.002	0.003 ± 0.000	1.311 ± 0.159
Plasma	2.328 ± 0.114	0.021 ± 0.001	0.006 ± 0.000	0.153 ± 0.009
Prostate	3.238 ± 0.370	0.028 ± 0.003	0.008 ± 0.001	0.000 ± 0.000
Skin	7.579 ± 0.844	0.067 ± 0.007	0.018 ± 0.001	3.255 ± 0.260
Spleen	7.808 ± 0.804	0.069 ± 0.008	0.019 ± 0.002	0.039 ± 0.002
Testes	6.805 ± 0.379	0.060 ± 0.004	0.016 ± 0.001	0.000 ± 0.000
Thyroid	1571.119 ± 330.362	13.693 ± 2.508	3.741 ± 0.717	0.281 ± 0.054
Urinary Bladder	4.266 ± 0.304	0.038 ± 0.003	0.010 ± 0.001	0.000 ± 0.000

NM-412 14 Day (n=4)	dpm/mg	% dose/gm ± SEM	% kg-does/gm ± SEM	% dose/organ ± SEM
Adrenal	16.812 ± 1.826	0.149 ± 0.011	0.041 ± 0.002	0.010 ± 0.000
Blood	0.646 ± 0.085	0.006 ± 0.001	0.002 ± 0.000	0.080 ± 0.012
Bone Marrow	4.763 ± 0.802	0.042 ± 0.005	0.011 ± 0.001	0.040 ± 0.003
Duodenum	1.787 ± 0.288	0.016 ± 0.002	0.004 ± 0.000	0.083 ± 0.006
Eye	0.869 ± 0.107	0.008 ± 0.001	0.002 ± 0.000	0.003 ± 0.000
Fat	15.230 ± 1.599	0.135 ± 0.007	0.037 ± 0.003	2.651 ± 0.184
Heart	0.976 ± 0.059	0.009 ± 0.000	0.002 ± 0.000	0.007 ± 0.000
Kidney	2.471 ± 0.443	0.022 ± 0.003	0.006 ± 0.001	0.045 ± 0.005
Liver	3.116 ± 0.192	0.028 ± 0.002	0.008 ± 0.001	0.295 ± 0.012
Lung	2.406 ± 0.102	0.021 ± 0.000	0.006 ± 0.000	0.033 ± 0.001
Muscle	0.717 ± 0.188	0.007 ± 0.002	0.002 ± 0.001	0.823 ± 0.241
Plasma	0.677 ± 0.069	0.006 ± 0.000	0.002 ± 0.000	0.045 ± 0.004
Prostate	1.156 ± 0.223	0.010 ± 0.001	0.003 ± 0.000	0.000 ± 0.000
Skin	4.268 ± 0.573	0.038 ± 0.004	0.011 ± 0.002	1.911 ± 0.284
Spleen	4.870 ± 0.334	0.044 ± 0.004	0.012 ± 0.001	0.027 ± 0.002
Testes	6.048 ± 0.353	0.054 ± 0.002	0.015 ± 0.000	0.000 ± 0.000
Thyroid	787.892 ± 100.612	6.988 ± 0.705	1.935 ± 0.199	0.145 ± 0.015
Urinary Bladder	1.439 ± 0.156	0.013 ± 0.002	0.004 ± 0.000	0.000 ± 0.000

TABLE 2. PREDICTED DOSIMETRY TO MIRD ADULT PHANTOM OF ^{131}I-LABELED PHOSPHOLIPID ETHER ANALOGS BASED UPON RAT BIODISTRIBUTION DATA.

TARGET ORGAN	NM-324 rad/mCi	NM-404 rad/mCi	NM-412 rad/mCi
Adrenals	0.646	2.270	1.650
Brain	0.014	0.057	0.018
Breasts	0.069	0.146	0.048
Gallbladder	0.466	0.367	0.145
LLI Wall	0.239	0.259	0.078
Small Intestine	4.070	0.243	0.078
Stomach	0.197	0.265	0.088
ULI Wall	0.507	0.245	0.081
Heart Wall	0.401	1.100	0.289
Kidneys	4.200	1.850	0.701
Liver	2.360	1.260	0.689
Lungs	0.838	2.080	0.697
Muscle	0.229	0.887	0.258
Pancreas	0.281	0.360	0.129
Red Marrow	0.153	0.889	0.335
Bone Surfaces	0.121	0.613	0.222
Skin	0.063	0.137	0.042
Spleen	0.826	1.520	0.696
Testes	0.063	1.430	0.358
Thymus	0.099	0.267	0.082
Thyroid	0.069	3.040	0.068
Urinary Bladder	0.134	0.254	0.074
Total Body	0.318	0.557	0.178

Residence Times: (source organs used)

	NM-324	NM-404	NM-412
Adrenals	0.013	0.073	0.058
Small Intestine	70510		
Heart Wall	0.171	0.575	0.135
Kidneys	2.570	1.020	0.391
Liver	7.940	3.780	2.230
Lungs	1.610	4.170	1.400
Muscle	13.10	45.00	12.80
Red Marrow		3.430	1.360
Spleen	0.252	0.490	0.239
Testes		0.110	0.027
Thyroid		0.135	

Appendix 4: Acute Toxicology of NM-404 in Rats and Rabbits

University at Buffalo
State University of New York

Toxicology Research Center

April 5, 1999

Dr, Ray Counsell
University of Michigan
Medical School
Department of Pharmacy
Ann Arbor, MI 48109-0632

Dear Dr. Counsell:

Enclosed please find the final reports for the acute toxicity assays in rat and rabbit for NM-404. At the dose studied, there were no pathological changes attributable to the test article in comparing test animals with controls. Slight differences in chemical, hematological or organ weights between test and control animals were not consistent across species. At the dose used, there were no apparent signs of distress in the animals throughout the observation period. The reports include the protocols utilized, and a detailed compilation of the data collected.

Thank you for the opportunity to evaluate this new radio-imaging agent.

Very truly yours,

Dr. Paul J. Kostyniak

Farber Hall Rm. 111, 3435 Main St., Buffalo, NY 14214-3000
Tel: (716) 829-2125 Fax: (716) 829-2806

STUDY 27 – FINAL REPORT

NM-404

Acute Toxicology Study in the Rat

The purpose of this study was to evaluate the toxicity in rats of phospholipid ether NM-404 (alkyl chain length of 18 carbons), a radioimaging agent for tumors. The control and test articles were formulated by Raymond E. Counsell, Ph.D., Professor of Pharmacology & Medicinal Chemistry, Department of Pharmacology, The University of Michigan Medical School, Ann Arbor, Michigan. The test article was NM-404 in a solution of 2% Tween 20 and sterile water. The control article was only 2% Tween 20 and sterile water. The sponsor of this project was responsible for the specifications of the test and control articles with concern for contaminants that could reasonably be expected to be present and capable of interfering with the purpose of this study. All procedures followed during this study are included in "Study 27 – Protocol" which is attached at the end of this report.

The control and test articles were received from Dr. Counsell on October 29, 1998. At the University of Buffalo, the study test site, the four (4) vials of test articles labeled "NM-404 in 2% Tween 20/Sterile Water, MAL-V1-82" and the four (4) vials of control articles labeled "Control Vehicle – 2% Tween 20/Sterile Water, MAL-V1-83" were inventoried and stored at room temperature in Farber Hall, Room 111. All the vials were dated October 16, 1998. The test article of the NM-404 solution was to be administered at approximately 200 times the clinical dose at a concentration of 2 mg/ml and a dose of 4 mg/kg. Each vial was reported to have an approximate volume of 10 ml. A green sticker dot was attached to each test vial to designate the NM-404 solution from the control solution; the control vials were designated with a "C" and then each set of control and test vials were numbered #1 to #4. Control vial #1C and test vial (with a green dot) #1 were injected on December 2, 1998 (Day 0 of the study). The control and test rats were injected intravenously in the lateral tail veins at a dose of 2 ml/kg.

On November 24, 1998 sixteen (16) Sprague-Dawley rats were received from Harlan Sprague Dawley, Indianapolis, Indiana. The rats were all males, all born on October 9, 1998, and all appeared healthy. They were housed at the Laboratory Animal Facility, CFS Addition – Room 110E. The next day the rats were weighed and their weights ranged from 223.6 grams to 250.7 grams. Two groups of eight (8) rats per group, controls and tests, were established with a mean weight for each group of 238.4 grams and 234.0 grams, respectively. The rats were housed two (2) animals per cage and given food and water ad lib. Each rat was ear punched with a unique number of '1' to '16', numerically. The control rats were numbered '1' to '8' and the test rats were numbered '9' to '16'. The unique numbers were also applied to each cage indicating

which rats were housed within. There were four cages of control rats and four cages of test rats. The rats were then weighed daily Monday through Friday until the termination of the study.

On December 2, 1998 (Day 0) the eight (8) control and eight (8) test rats were placed in a commercial rat restrainer, their tails scrubbed with alcohol, and then the tail placed in a container of very warm water for a minute to dialate the tail vein. The rats were injected intravenously in the lateral tail vein at 2 ml/kg of body weight using a 25 gauge needle and a 1 ml syringe. The injections were given by alternating a rat from the control group with a rat from the test group, with the injections given over a 30 second to 1 minute interval. Control rat #6 received the injection in 2 sites; test rat #9 received the injection in 2 sites and test rat #15 received the injection in 3 sites. This occurred because the rat moved during the injection procedure. All other injections were given at one site only. The injection on control rat #1 was given at 9:03 A.M. and the last injection of test rat #16 was given at 11:01 A.M. No adverse reactions were observed at the time of the injection or noted after the injections were completed. The rats were observed for signs of acute toxicity as described in Principles and Methods of Toxicology, 2nd Edition, Editor: A.W. Hayes, 1989, p. 180-181. The rats were observed closely until 1:15 P.M., and again at 3:30 P.M. No unusual behavior was noted in any of the rats during this time or during the remainder of the study. The tail injection sites were observed daily while the rats were weighed and no adverse tissue reactions were noted in any of the rats.

The rats were weighed five (5) times a week (Monday through Friday) and their weights, recorded in kilograms, appear in Table 1. The mean weights of the control group and the test group also appear in Table 1 and these values are compared in Graph 1. Weight gain appears to be consistent between the two groups.

The rats were anesthetized with sodium pentobarbital administered intraperitoneally (65 mg/ml, Lot #970789, Expiration Date: 2/00) on December 17, 1998. A heart puncture was then performed using a 20 gauge needle and a 10 ml syringe to collect the blood samples for hematology testing. The rats were exsanguinated to cause death. The brain, heart, lungs, thymus, spleen, kidneys (both), liver, and testes (both) were collected, examined grossly, weighed, and sectioned for pathology. The organ weights and the organ to final body weight ratio data appears in Table 2. The organ/body weight ratios were compared using a Students t-test and the only significant difference was found between the lung/body weight ratio of the study control and test rats. The tissue samples (except thymus) were placed in jars of 'Z-Fix' fixative. The following week, the organs were examined by the pathologist, Dr. Peter Nickerson, and cut into a representative section for processing for histopathological examination. Dr. Nickerson reports that "no gross lesions were noted in any of the groups receiving either the test material or in the control group". The collected organs were processed by the Department of Pathology, SUNY at Buffalo, School of Medicine. The histological preparations were examined by Peter A. Nickerson, Ph.D., Professor of Pathology, SUNY at Buffalo. The report from Dr. Nickerson lists his findings for control rat #27-01 to #27-08 and test rat #27-09 to #27-16, with a description of each organ. Dr. Nickerson

concludes that "by light microscopic examination, there are no changes in the histopathology of the organs examined that can be attributed to administration of the test material. Rat #12 receiving the test material has a small, focal area of myocardial injury (Infarct in an early stage). This change is not seen in the other section of heart that was also processed so is interpreted as being quite small. Since the lesion is not seen in other animals receiving the material, it is likely that it is attributable to some unexplained alteration. It is not due to infection arising from the lung since the lungs did not show histopathological change". The complete histological report prepared by Dr. Nickerson is attached.

Clinical blood chemistries (Superchem) and a CBC (Complete Blood Count with differential) were performed by Antech Diagnostics, Memphis, Tennessee on December 17, 1998 (Day 14). The results of the Superchem screen and CBC appear in Table 3 (Page 1: Control Rats #1 - #8; Page 2: Test Rats #9 - #16; Page 3: Mean Data with Standard Deviation for both groups). The values were checked for any obvious low or high values that were below or above the reference range provided by Antech Diagnostics. Whenever any value was outside of this reference range the groups were compared using a Students t-test at a p value of less than 0.05. T-tests were performed for the following blood tests: Phosphorus, Sodium, Potassium, AST (SGOT), ALT (SGPT), Alkaline phosphatase, Globulin, A/G ratio, Glucose, WBC, RBC, and Hemoglobin. There were no significant differences found. The platelet estimation was similar between the control and test rats (Control rats: adequate in 1 rat, increased in 5 rats, decreased in 2 rats; Test rats: adequate in 3 rats, increased in 5 rats, decreased in no rats). Platelets were found to be clumped in 3 control rats and 2 test rats. Slight anistocytosis was reported in 3 control rats and 6 test rats; only moderate anistocytosis was reported in 1 control rat. Slight polychromasia was reported in 6 control rats and 6 test rats. Only control rat #27-07 was reported to have 1 NRBC/100 WBC.

External factors which might effect the study outcome appear to have been very limited in this study. Daily observations of the rats were also made by the personnel of the Laboratory Animal Facilities, SUNY at Buffalo.

At the conclusion of this study, all data notebooks with reports, histology tissue blocks, and stained slides are stored in Farber Hall, Room 118G at SUNY at Buffalo. All materials are labeled as "Study 27".

During the course of this study, an attempt was made to follow the Good Laboratory Practice (GLP) Regulations as outlined in the Federal Register of December 22, 1978 and September 4, 1987, Final Rule (21 CFR Part 58). Technical procedures are described in the "Study 27 – Protocol NM-404 Acute Toxicology Study in the Rat" which is attached at the end of this report.

At the State University of New York at Buffalo, the Study Technician was Ellen M. Schopp under the supervision of the Project Director, Paul J. Kostyniak, Ph.D. A special thanks to Marian M. Pazik for his assistance and cooperation during this study and for his technical support in the compiling of the study data and to Joseph A.

Syracuse, Ph.D. for his support on the final day. The Quality Assurance Officer who reviewed this report was Hebe B. Greizerstein, Ph.D.

Reviewed and Approved by:

Dr. Paul J. Kostyniak
Study Director

3-24-99
Date

Ellen M. Schopp
Ellen M. Schopp
Study Technician

3/23/99
Date

Dr. Hebe B. Greizerstein
Quality Assurance Officer

3/23/99
Date

STUDY # 27

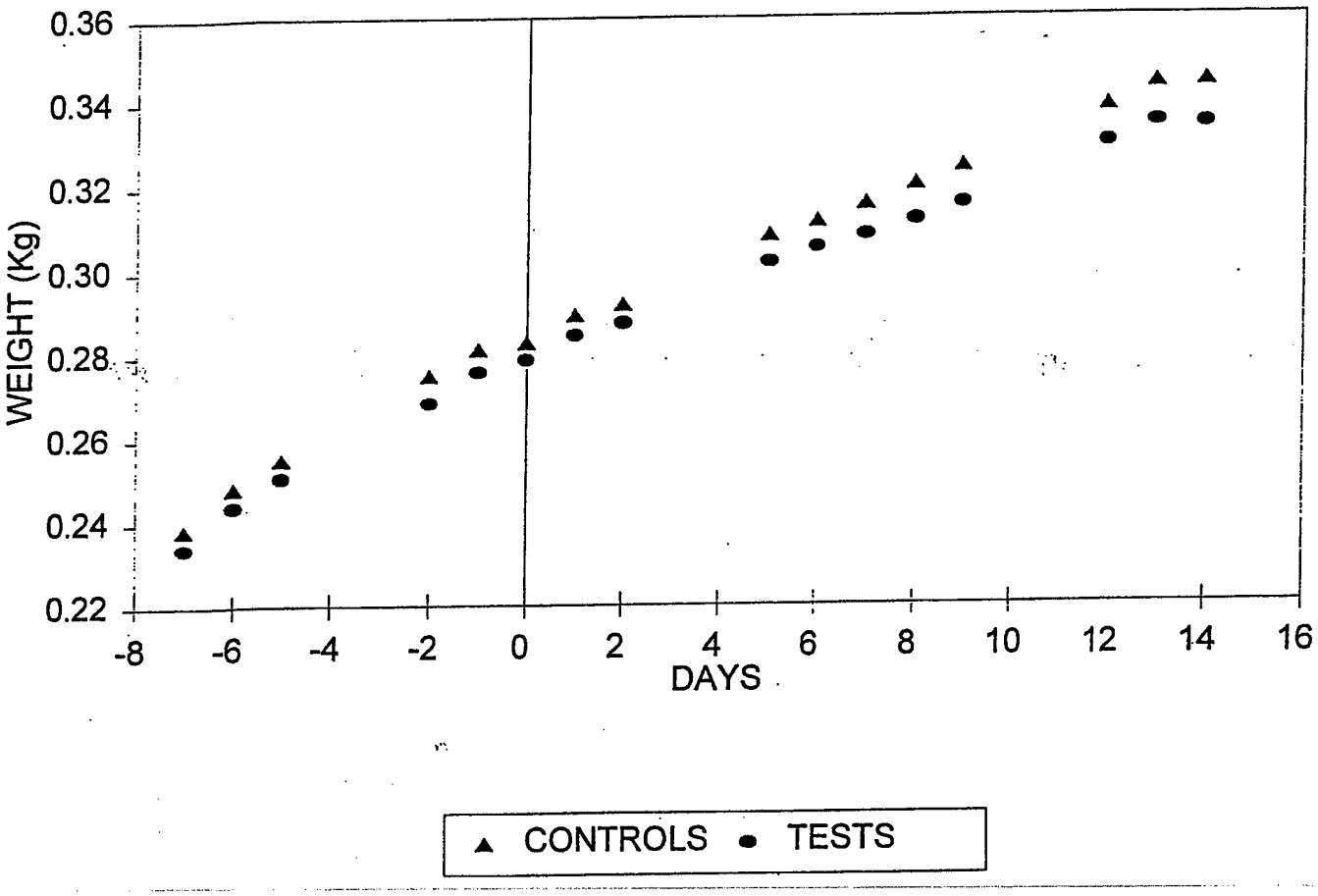
DESCRIPTION : NM-404: Rat /Acute

RAT WEIGHTS (Kilograms)

DAY	-9	-8	-7	-6	-5	-2	-1	0	1	2	5	6	7	8	9	12	13	14
CONTROLS																		
27-01			0.224	0.231	0.239	0.271	0.276	0.280	0.284	0.289	0.303	0.305	0.311	0.314	0.315	0.330	0.337	0.339
27-02			0.235	0.241	0.246	0.258	0.272	0.267	0.272	0.275	0.295	0.300	0.302	0.304	0.308	0.323	0.325	0.328
27-03			0.243	0.256	0.263	0.289	0.294	0.299	0.306	0.308	0.328	0.334	0.338	0.341	0.351	0.360	0.365	0.369
27-04			0.238	0.246	0.247	0.264	0.265	0.265	0.271	0.272	0.283	0.283	0.286	0.290	0.289	0.304	0.308	0.307
27-05			0.248	0.258	0.264	0.281	0.286	0.288	0.296	0.298	0.314	0.320	0.324	0.330	0.333	0.348	0.357	0.362
27-06			0.251	0.263	0.273	0.294	0.299	0.301	0.310	0.312	0.328	0.329	0.336	0.342	0.347	0.359	0.366	0.364
27-07			0.232	0.241	0.251	0.272	0.281	0.284	0.294	0.296	0.315	0.319	0.323	0.329	0.333	0.352	0.355	0.353
27-08			0.237	0.250	0.257	0.272	0.277	0.279	0.282	0.286	0.300	0.302	0.306	0.312	0.319	0.332	0.339	0.333
AVG			0.24	0.25	0.26	0.28	0.28	0.28	0.29	0.29	0.31	0.31	0.32	0.32	0.32	0.34	0.34	0.34
STD			0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02
MEAN+STD			0.25	0.26	0.27	0.29	0.29	0.30	0.30	0.31	0.32	0.33	0.33	0.34	0.34	0.36	0.36	0.36
MEAN-STD			0.23	0.24	0.24	0.26	0.27	0.27	0.28	0.28	0.29	0.30	0.30	0.30	0.30	0.32	0.32	0.32
TESTS																		
27-09			0.225	0.235	0.242	0.259	0.268	0.271	0.275	0.280	0.296	0.306	0.304	0.308	0.313	0.326	0.328	0.333
27-10			0.233	0.243	0.249	0.270	0.277	0.280	0.286	0.287	0.305	0.302	0.310	0.315	0.316	0.327	0.329	0.329
27-11			0.239	0.246	0.253	0.276	0.282	0.286	0.293	0.297	0.311	0.315	0.324	0.326	0.331	0.346	0.353	0.350
27-12			0.228	0.239	0.248	0.262	0.271	0.273	0.279	0.281	0.296	0.296	0.297	0.300	0.304	0.314	0.318	0.317
27-13			0.245	0.251	0.259	0.280	0.280	0.285	0.291	0.292	0.304	0.309	0.311	0.312	0.315	0.328	0.334	0.330
27-14			0.243	0.254	0.259	0.277	0.285	0.286	0.293	0.296	0.308	0.312	0.316	0.321	0.324	0.344	0.349	0.346
27-15			0.232	0.241	0.247	0.260	0.273	0.277	0.281	0.284	0.296	0.298	0.301	0.306	0.308	0.326	0.332	0.334
27-16			0.227	0.241	0.248	0.264	0.271	0.273	0.278	0.283	0.298	0.304	0.302	0.306	0.313	0.327	0.333	0.334
AVG			0.23	0.24	0.25	0.27	0.28	0.28	0.28	0.29	0.30	0.31	0.31	0.31	0.32	0.33	0.33	0.33
STD			0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
MEAN+STD			0.24	0.25	0.26	0.28	0.28	0.28	0.29	0.29	0.31	0.31	0.32	0.32	0.32	0.34	0.35	0.34
MEAN-STD			0.23	0.24	0.25	0.26	0.27	0.27	0.28	0.28	0.30	0.30	0.30	0.30	0.31	0.32	0.32	0.32

Table 1

STUDY 27: NM-404
Rats - Acute



STUDY # 27

DESCRIPTION: NM-404: Rats / Acute

ORGAN WEIGHTS AND ORGAN/BODY WEIGHT RATIOS

CONTROLS	Final Body Weight (kg)	Final Body Weight (kg)	Brain (g)	Brain/BW Ratio	Testes (g)	Testes/BW Ratio	Liver (g)	Liver/BW Ratio	Kidneys (g)	Kidney/BW Ratio
27-01	0.339	0.339	1.70	5.015	3.74	11.032	13.15	38.791	2.20	6.490
27-02	0.328	0.328	1.82	5.549	3.80	11.585	12.87	39.238	2.25	6.860
27-03	0.369	0.369	1.85	5.014	4.03	10.921	14.19	38.455	2.39	6.477
27-04	0.307	0.307	1.70	5.537	3.64	11.857	10.47	34.104	1.93	6.287
27-05	0.362	0.362	1.83	5.055	3.63	10.028	13.51	37.320	2.18	6.022
27-06	0.364	0.364	1.72	4.725	3.56	9.780	13.22	36.319	2.02	5.549
27-07	0.353	0.353	1.91	5.411	3.86	10.935	12.93	36.629	2.28	6.459
27-08	0.333	0.333	1.65	4.955	3.13	9.399	12.90	38.739	2.06	6.186
Mean	0.344	0.344	1.773	5.158	3.674	10.692	12.905	37.449	2.164	6.291
STD	0.020	0.020	0.086	0.283	0.248	0.816	1.007	1.614	0.141	0.364
Mean + STD	0.364	0.364	1.86	5.44	3.92	11.51	13.91	39.06	2.31	6.66
Mean - STD	0.324	0.324	1.69	4.87	3.43	9.88	11.90	35.84	2.02	5.93
TESTS										
27-09	0.333	0.333	1.72	5.165	3.40	10.210	11.73	35.225	2.08	6.246
27-10	0.329	0.329	1.81	5.502	3.80	11.550	11.54	35.076	2.20	6.687
27-11	0.350	0.350	1.80	5.143	3.67	10.486	13.21	37.743	2.21	6.314
27-12	0.317	0.317	1.80	5.678	3.81	12.019	12.72	40.126	2.31	7.287
27-13	0.330	0.330	1.76	5.333	3.48	10.545	11.97	36.273	2.12	6.424
27-14	0.346	0.346	1.82	5.260	3.83	11.069	12.34	35.665	2.18	6.301
27-15	0.334	0.334	1.77	5.299	3.73	11.168	11.73	35.120	2.04	6.108
27-16	0.334	0.334	1.79	5.359	3.48	10.419	12.28	36.766	1.95	5.838
Mean	0.334	0.334	1.784	5.342	3.650	10.933	12.190	36.499	2.136	6.401
STD	0.010	0.010	0.030	0.165	0.161	0.588	0.530	1.623	0.105	0.405
Mean + STD	0.344	0.344	1.81	5.51	3.81	11.52	12.72	38.12	2.24	6.81
Mean - STD	0.325	0.325	1.75	5.18	3.49	10.34	11.66	34.88	2.03	6.00

Table 2

STUDY # 27

DESCRIPTION: NM-404: Rats / Acute

ORGAN WEIGHTS AND ORGAN/BODY WEIGHT RATIOS

CONTROLS	Final Body Weight (kg)	Spleen (g)	Spleen/BW Ratio	Heart (g)	Heart/BW Ratio	Lungs (g)	Lung/BW Ratio	Thymus (g)	Thymus/BW Ratio
27-01	0.339	0.69	2.035	1.14	3.363	1.28	3.776	0.64	1.888
27-02	0.328	0.71	2.165	1.19	3.628	1.33	4.055	0.40	1.220
27-03	0.369	0.91	2.466	1.14	3.089	1.47	3.984	0.58	1.572
27-04	0.307	0.80	2.606	1.07	3.485	1.28	4.169	0.39	1.270
27-05	0.362	0.82	2.265	1.15	3.177	1.37	3.785	0.35	0.967
27-06	0.364	0.81	2.225	1.16	3.187	1.34	3.681	0.59	1.621
27-07	0.353	0.84	2.380	1.25	3.541	1.23	3.484	0.51	1.445
27-08	0.333	0.84	2.523	1.25	3.754	1.34	4.024	0.47	1.411
Mean	0.344	0.803	2.333	1.169	3.403	1.330	3.870	0.491	1.424
STD	0.020	0.067	0.181	0.056	0.223	0.067	0.212	0.099	0.262
Mean + STD	0.364	0.87	2.51	1.23	3.63	1.40	4.08	0.59	1.69
Mean - STD	0.324	0.74	2.15	1.11	3.18	1.26	3.66	0.39	1.16
TESTS									
27-09	0.333	0.81	2.432	1.06	3.183	1.35	4.054	0.46	1.381
27-10	0.329	0.74	2.249	1.15	3.495	1.49	4.529	0.40	1.216
27-11	0.350	0.79	2.257	1.24	3.543	1.50	4.286	0.69	1.971
27-12	0.317	0.81	2.555	1.02	3.218	1.38	4.353	0.46	1.451
27-13	0.330	0.75	2.273	1.22	3.697	1.48	4.485	0.50	1.515
27-14	0.346	0.88	2.543	1.26	3.642	1.46	4.220	0.41	1.185
27-15	0.334	0.96	2.874	0.92	2.754	1.34	4.012	0.45	1.347
27-16	0.334	0.71	2.126	1.31	3.922	1.51	4.521	0.45	1.347
Mean	0.334	0.806	2.414	1.148	3.432	1.439	4.307	0.478	1.427
STD	0.010	0.076	0.225	0.127	0.342	0.066	0.190	0.085	0.230
Mean + STD	0.344	0.88	2.64	1.27	3.77	1.50	4.50	0.56	1.66
Mean - STD	0.325	0.73	2.19	1.02	3.09	1.37	4.12	0.39	1.20

Table 3

STUDY # 27

TITLE: NM-404: Rats / Acute

Blood Chemistry & CBC Results -

DAY # 14

ANIMAL # / BLOOD TEST	CONTROLS							
	27-01	27-02	27-03	27-04	27-05	27-06	27-07	27-08
Calcium mg/dL	11.6	10.7	10.6	10.7	10.4	10.1	10.4	10.3
Phosphorus mg/dL	8.0	10.5	8.4	8.6	9.5	8.1	8.3	9.1
Sodium mEq/L	142	142	139	142	139	143	142	141
Potassium mEq/L	5.4	5.4	4.2	5.0	4.7	4.3	4.5	5.0
Chloride mEq/L	103	100	99	101	98	102	103	101
Cholesterol mg/dL	97	110	86	86	82	85	91	104
Triglycerides mg/dL	147	21	127	57	54	125	148	93
AST (SGOT) U/L	96	125	95	209	210	93	99	101
Bilirubin, Total mg/dL	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
GGTP U/L	2	2	2	2	2	2	2	2
ALT (SGPT) U/L	68	70	65	67	59	47	50	92
Alkal. Phosphatase U/L	381	279	301	210	370	267	284	307
Protein, Total g/dL	6.3	5.7	5.9	5.8	5.5	5.5	5.8	5.5
Globulin g/dL	2.7	2.5	2.7	2.7	2.4	2.4	2.6	2.5
Albumin g/dL	3.6	3.2	3.2	3.1	3.1	3.1	3.2	3.0
A/G Ratio	1.30	1.30	1.20	1.10	1.30	1.30	1.20	1.20
Urea Nitrogen mg/dL	16	17	16	17	18	14	15	16
Creatinine mg/dL	0.8	0.9	0.8	0.9	1.0	0.9	0.8	0.9
BUN/Creatinine Ratio	20	19	20	19	18	16	19	18
Glucose mg/dL	163	212	214	226	265	199	222	193
Amylase U/L	3138	2908	2859	2811	2698	2510	2840	2911
Lipase U/L	6	4	7	5	6	7	7	9
CPK U/L	1008	2476	1405	4025	5445	1312	795	1352
Magnesium mEq/ L	1.9	3.3	2.0	2.2	2.6	2.0	2.2	2.3
Osmolality, Calc'd mosm/	289	292	284	292	288	290	290	288
WBC thds/cmm	3.6	1.4	4.7	4.2	6.7	5.4	6.4	3.3
RBC mill/cmm	7.74	6.80	7.29	5.36	6.79	7.14	6.94	7.15
Hemoglobin g/dL	15.3	13.6	14.3	13.8	13.2	14.1	14.0	14.6
Hematocrit %	46.4	43.4	44.4	31.8	40.8	42.8	41.7	45.1
MCV	60	64	61	59	60	60	60	63
MCH	19.8	20.0	19.6	25.7	19.4	19.7	20.2	20.4
MCHC	33.0	31.3	32.2	43.4	32.4	32.9	33.6	32.4
Polys %	10	7	22	4	8	23	13	11
Bands %	1	0	0	0	0	0	0	0
Lymphocytes %	79	93	66	92	83	70	87	77
Monocytes %	10	0	9	0	8	3	0	9
Eosinophils %	0	0	2	0	1	2	0	1
Basophils %	0	0	1	0	0	2	0	2
Platelet Estimation	Adequat	Increase	Increase	Decreas	Increase	Increase	Increase	Decreas
Platelet Comments		Clumps	Clumps				Clumps	
Anisocytosis	Slight	Slight		Slight		Moderat		
Polychromasia	Slight	Slight	Slight	Slight		Slight		Slight
Other Comments							1 NRBC	

STUDY # 27

TITLE: NM-404: Rats / Acute

Blood Chemistry & CBC Results - DAY # 14

ANIMAL # / BLOOD TEST	TESTS							
	27-09	27-10	27-11	27-12	27-13	27-14	27-15	27-16
Calcium mg/dL	10.1	10.8	11.4	10.1	10.7	10.6	9.9	10.5
Phosphorus mg/dL	9.4	10.6	8.9	8.3	10.4	10.0	9.3	8.5
Sodium mEq/L	142	141	141	138	140	141	143	145
Potassium mEq/L	4.5	5.5	4.9	4.3	5.7	5.6	4.4	4.9
Chloride mEq/L	101	100	100	100	99	99	103	104
Cholesterol mg/dL	103	102	89	70	91	96	60	90
Triglycerides mg/dL	85	77	65	119	95	123	89	161
AST (SGOT) U/L	95	328	149	103	95	130	127	106
Bilirubin, Total mg/dL	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
GGTP U/L	2	2	2	2	2	2	2	2
ALT (SGPT) U/L	45	62	77	49	52	50	52	57
Alkal. Phosphatase U/L	290	277	279	273	192	231	277	242
Protein, Total g/dL	5.8	5.9	5.5	5.5	5.8	5.9	5.5	5.5
Globulin g/dL	2.6	2.6	2.3	2.3	2.4	2.6	2.5	2.4
Albumin g/dL	3.2	3.3	3.2	3.2	3.4	3.3	3.0	3.1
A/G Ratio	1.20	1.30	1.40	1.40	1.40	1.30	1.20	1.30
Urea Nitrogen mg/dL	18	18	18	18	21	16	16	16
Creatinine mg/dL	0.8	0.9	0.9	0.8	0.9	1.0	0.9	0.9
BUN/Creatinine Ratio	23	20	20	23	23	16	18	18
Glucose mg/dL	190	200	206	200	243	236	197	206
Amylase U/L	2768	2932	2385	2466	2772	3405	2318	3348
Lipase U/L	6	5	5	5	6	6	6	6
CPK U/L	721	4840	2681	1355	1233	2004	2011	1142
Magnesium mEq/ L	2.2	2.9	2.3	2.0	2.9	2.7	1.9	2.4
Osmolality, Calc'd mosm/	289	290	289	282	292	292	291	296
WBC thds/cmm	3.6	4.2	3.4	3.7	5.9	1.7	6.1	4.9
RBC mill/cmm	7.08	6.95	6.93	7.47	7.45	7.28	7.46	7.56
Hemoglobin g/dL	13.5	13.4	13.4	14.3	14.9	13.9	13.8	14.7
Hematocrit %	42.7	43.1	42.2	43.1	45.6	43.7	43.8	45.0
MCV	60	62	61	58	61	60	59	60
MCH	19.1	19.3	19.3	19.1	20.0	19.1	18.5	19.4
MCHC	31.6	31.1	31.8	33.2	32.7	31.8	31.5	32.7
Polys %	10	22	7	20	13	27	7	20
Bands %	0	0	0	0	0	0	0	0
Lymphocytes %	88	70	92	72	86	67	93	75
Monocytes %	1	4	1	5	1	2	1	3
Eosinophils %	1	2	0	1	1	2	1	1
Basophils %	4	2	0	2	3	2	2	1
Platelet Estimation	Adequat	Adequat	Increase	Increase	Increase	Increase	Increase	Adequat
Platelet Comments	Clumps				Clumps			
Anisocytosis	Slight	Slight	Slight	Slight	Slight	Slight		
Polychromasia	Slight		Slight	Slight	Slight	Slight	Slight	
Other Comments								

STUDY # 27

TITLE: NM-404: Rats / Acute

Blood Chemistry & CBC Results - DAY # 14

ANIMAL # / BLOOD TEST	MEAN DATA CONTROLS MEAN	CONTROLS STD	TESTS MEAN	TESTS STD	* Significant Difference
Calcium mg/dL	10.6	0.4	10.5	0.5	
Phosphorus mg/dL	8.8	0.8	9.4	0.8	
Sodium mEq/L	141.3	1.4	141.4	1.9	
Potassium mEq/L	4.8	0.4	5.0	0.5	
Chloride mEq/L	100.9	1.7	100.8	1.7	
Cholesterol mg/dL	92.6	9.4	87.6	14.2	
Triglycerides mg/dL	96.5	44.8	101.8	29.0	
AST (SGOT) U/L	128.5	47.7	141.6	72.7	
Billirubin, Total mg/dL	0.1	0.0	0.1	0.0	
GGTP U/L	2.0	0.0	2.0	0.0	
ALT (SGPT) U/L	64.8	13.0	55.5	9.4	
Alkal. Phosphatase U/L	299.9	51.7	257.6	31.1	
Protein, Total g/dL	5.8	0.3	5.7	0.2	
Globulin g/dL	2.6	0.1	2.5	0.1	
Albumin g/dL	3.2	0.2	3.2	0.1	
A/G Ratio	1.2	0.1	1.3	0.1	
Urea Nitrogen mg/dL	16.1	1.2	17.6	1.6	
Creatinine mg/dL	0.9	0.1	0.9	0.1	
BUN/Creatinine Ratio	18.6	1.2	20.1	2.5	
Glucose mg/dL	211.8	27.5	209.8	17.9	
Amylase U/L	2834.4	169.0	2799.3	387.6	
Lipase U/L	6.4	1.4	5.6	0.5	
CPK U/L	2227.3	1559.9	1998.4	1219.9	
Magnesium mEq/ L	2.3	0.4	2.4	0.4	
Osmolality, Calc'd mosm/	289.1	2.4	290.1	3.7	
WBC thds/cmm	4.5	1.6	4.2	1.3	
RBC mill/cmm	6.9	0.6	7.3	0.2	
Hemoglobin g/dL	14.1	0.6	14.0	0.5	
Hematocrit %	42.1	4.2	43.7	1.1	
MCV	60.9	1.6	60.1	1.2	
MCH	20.6	2.0	19.2	0.4	
MCHC	33.9	3.6	32.1	0.7	
Polys %	12.3	6.4	15.8	7.0	
Bands %	0.1	0.3	0.0	0.0	
Lymphocytes %	80.9	9.2	80.4	9.8	
Monocytes %	4.9	4.3	2.3	1.5	
Eosinophils %	0.8	0.8	1.1	0.6	
Basophils %	0.6	0.9	2.0	1.1	
Platelet Estimation					
Platelet Comments					
Anisocytosis					
Polychromasia					
Other Comments					

STUDY 27

Gross Examination:

Organs were examined grossly at the time of removal and after fixation, before a representative section was cut for processing for histopathological examination. No gross lesions were noted in any of the groups receiving either the test material or in the control group.

Histopathological Examination of Organs:

Rats receiving the Test Material.

Rat #9

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

~~Spleen: White and red pulp have normal structure.~~

Testis: The testis is mature, spermatogenesis is normal and there are spermatozoa in the seminiferous tubules. The epididymis is normal.

Rat #10

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp have normal structure.

Testis: The testis is mature, spermatogenesis is normal and there are spermatozoa in the seminiferous tubules. The epididymis is normal.

Rat #11

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp have normal structure.

Testis: The testis is mature, spermatogenesis is normal and there are spermatozoa in the seminiferous tubules. The epididymis is normal.

Rat #12

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: There is a focal area in the myocardium that extends to the endocardium which contains lightly eosinophilic myocardium and numerous macrophages. The other half of the heart was also processed for histology and did not contain this lesion; coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp have normal structure.

Testis: The testis is mature, spermatogenesis is normal and there are spermatozoa in the seminiferous tubules. The epididymis is normal.

Rat #13

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp have normal structure.

Testis: The testis is mature, spermatogenesis is normal and there are spermatozoa in the seminiferous tubules. The epididymis is normal.

Rat #14

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp have normal structure.

Testis: The testis is mature, spermatogenesis is normal and there are spermatozoa in the seminiferous tubules. The epididymis is normal.

Rat #15

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of

infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp have normal structure.

Testis: The testis is mature, spermatogenesis is normal and there are spermatozoa in the seminiferous tubules. The epididymis is normal.

Rat #16

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp have normal structure.

Testis: The testis is mature, spermatogenesis is normal and there are spermatozoa in the seminiferous tubules. The epididymis is normal.

Control Group:

Rat #1

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp have normal structure.

Testis: The testis is mature, spermatogenesis is normal and there are spermatozoa in the seminiferous tubules. The epididymis is normal.

Rat #2

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp have normal structure.

Testis: The testis is mature, spermatogenesis is normal and there are spermatozoa in the seminiferous tubules. The epididymis is normal.

Rat #3

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp have normal structure.

Testis: The testis is mature, spermatogenesis is normal and there are spermatozoa in the seminiferous tubules. The epididymis is normal.

Rat #4

Brain: The cerebrum and cerebellum have normal structure; there is

no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp have normal structure.

Testis: The testis is mature, spermatogenesis is normal and there are spermatozoa in the seminiferous tubules. The epididymis is normal.

Rat #5

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp have normal structure.

Testis: The testis is mature, spermatogenesis is normal and there are

spermatozoa in the seminiferous tubules. The epididymis is normal.

Rat #6

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp have normal structure.

Testis: The testis is mature, spermatogenesis is normal and there are spermatozoa in the seminiferous tubules. The epididymis is normal.

Rat #7

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp have normal structure.

Testis: The testis is mature, spermatogenesis is normal and there are spermatozoa in the seminiferous tubules. The epididymis is normal.

Rat #8

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp have normal structure.

Testis: The testis is mature, spermatogenesis is normal and there are spermatozoa in the seminiferous tubules. The epididymis is normal.

Interpretation and comments:

By light microscopic examination, there are no changes in the histopathology of the organs examined that can be attributed to administration of the test material. Rat #12 receiving the test material has a small, focal area of myocardial injury (infarct in an early stage). This change is not seen in the other section of heart that was also

processed so is interpreted as being quite small. Since the lesion is not seen in other animals receiving the material, it is likely that it is attributable to some unexplained alteration. It is not due to infection arising from the lung since the lungs did not show histopathological change.

Peter A. Nickerson — 3/22/99

Peter A. Nickerson, Ph.D. — Date
Professor of Pathology

STUDY 27 - PROTOCOL

NM-404

ACUTE TOXICOLOGY STUDY

IN THE RAT

I. Description

The purpose of this study is to evaluate the toxicity in rats of phospholipid ether NM-404 (alkyl chain length of 18 carbons), a radioimaging agent for tumors.

II. Control/Test Articles

The formulation of the control and test articles will be performed by the sponsor. The control and test articles received from the sponsor for this study will be stored at room temperature in Farber Hall, Room 111, SUNY at Buffalo, New York. At termination of the study, the remaining control and test articles will be returned to the sponsor for QC and integrity testing.

Control Article: The control solution will be 2% Tween 20 and sterile water.

Test Article: The NM-404 solution will be NM-404 in a solution of 2% Tween 20 and sterile water. The test article of NM-404 solution will be approximately 200 times the clinical dose with a concentration of 2 mg/ml.

III. Sponsor/Testing Facility

Sponsor: Raymond E. Counsell, Ph.D.
Professor of Pharmacology & Medicinal Chemistry
Department of Pharmacology
1301 Medical Science Research Building
The University of Michigan Medical School
Ann Arbor, Michigan 48109-0632
Office: 313-764-8165
FAX: 313-763-4450

Project Director: Paul J. Kostyniak, Ph.D.
Director, Toxicology Research Center
Farber Hall - Room 111
SUNY at Buffalo
Office: 716-829-2125
FAX: 716-829-2806

Testing Facility: SUNY at Buffalo
Laboratory Animal Facilities
CFS Addition
Main Street Campus
Buffalo, New York 14214-3000

Laboratory Animal Facilities
Director: Thomas Martin, BVSC DipVetPath PhD MBA
MACVSc DiplACLAM
Laboratory Animal Facilities
116 CFS Addition
SUNY at Buffalo
Office: 716-829-2919
FAX: 716-829-3249

The Institutional Animal Care and Use Committee (IACUC) at the University of Buffalo has approved this study with the animal use project number of PMY22074N.

IV. Test System

Rat Supplier: Harlan Sprague Dawley
P.O. Box 29176
Indianapolis, Indiana 46229
1-800-793-7297

Rat Description: Sprague Dawley
Male
225-250 grams
Quantity - 16 animals

The rats will be housed at the State University of New York at Buffalo, Laboratory Animal Facilities, CFS Addition - Room 110 E.

V. Identification of Test System

Each rat will be given a two-part number starting with 27- (Study #), followed by a 'unique' number of '01' to '16' (numerical). The unique number will be applied to each rat using the ear punch identification code as illustrated in Figure 13-1, Manual for Assistant Laboratory Animal Technicians, W.B. Sapanski, Jr. & J.E. Harkness, Eds., August, 1984. The unique number will be applied to each rat using an animal ear punch. Each rat, housed two (2) animals per cage, will have a cage card with the unique numbers indicated and applied with a Sanford Sharpie Fine Point Permanent Marker, reflective of the rats housed within.

When referring to any rat during this study the 'unique' number of '01' to '16' will be referred to.

IV. Experimental Design

A. The sixteen (16) rats are randomly divided into two (2) groups having approximately the same mean weight:

1. Control Group: Eight (8) rats to receive the control article of 2% Tween 20

2. Test Group: Eight (8) rats to receive the test article of NM-404 in 2% Tween 20

B. The rats are weighed and their weights recorded in grams (g) during the one week acclimation period and during the two week study period, Monday through Friday, and more often if problems with weight gain occur.

C. The rats will be observed for any unusual behavior or change in food and water intake for the duration of the study.

D. The rats will be injected in one of the lateral tail veins, using sterile techniques, and an appropriately sized syringe with a 25 gauge needle (see Section VIII, Part B).

E. Initially, one (1) control and one (1) test rat will be injected at 2 ml/kg with the appropriate dosing solution:

1. These rats will be observed for any signs of toxicity, respiratory distress, change in motor activity, seizures, etc. (see Section IX, Part C).

   A. If any deaths occur, the remaining rats will be injected at 1/2 that dose rate (1 ml/kg) and observed.

   B. If no deaths occur, the remaining rats will be injected at the initial dose of 2 ml/kg, alternating control rat and test rat, and observed.

2. If any deaths occur following the injections, a veterinarian/pathologist will perform a post-mortem.

F. Fourteen (14) days after the dosing solution is injected, the rats will be killed:

1. Weigh the rat (final body weight).

2. Anesthetize the rat with the sodium pentobarbital, dosed 50 mg/kg with an intraperitoneal injection, using a 25 gauge needle and 1 ml syringe.

3. A heart puncture is then performed using a 20 gauge needle and 10 ml syringe to collect the blood samples for hematology testing (Superchem and CBC with differential) and to exsanguinate the rat causing death (see Section IX, Part D, #1).

4. Collect and examine grossly the following organs: Brain, Heart, Lungs, Thymus, Spleen, Kidneys (both), Liver and Testes (both) in the animal and upon removal.

5. Weigh each organ and record the weight (the weighing boats have been pre-weighed, their weights recorded and this weight needs to be subtracted from the combined organ and weighing boat weight to obtain organ weight).

6. Section organs (except thymus), if needed, for pathology (see Section IX, Part F, #1) and place the whole organ or representative organ sections in formalin.

7. Place carcass and remaining organs in plastic bag for incineration; clean area and instruments after each rat is sacrificed.

8. Prepare blood samples as described in Section IX, Part D, #2.

9. The organs and organ sections will be allowed to fix in the formalin for at least 24 hours before smaller sections are selected and cut to fit the histological cassettes for embedding.

10. Deliver the sectioned tissues in formalin to the Pathology Department, SUNY at Buffalo for histological preparation (See Section IX, Part F, #3).

VII. External Factors

A. Animal Diet

1. ProLab RMH 1000 Lab Diet

Guaranteed Analysis:

Crude protein not less than	14.0%
Crude fat not less than	6.0%
Crude fiber not more than	4.5%
Ash not more than	8.0%
Added minerals not more than	2.5%

Rats are fed ad lib

2. Water will be available from the automatic watering system that is attached to each cage rack, with a water spigot available the rats. The water is obtained from the City of Buffalo's public water system (tap water). The water spigot will be checked daily to assure that water is available to each cage.

B. Control and Test Articles

The sponsor of this project is responsible for the specifications of the control and test articles, with

concern for contaminants that could reasonably be expected to be present and capable of interfering with the purpose of this study.

VIII. Administration of Control/Test Articles

A. Dosage Level

The control and test rats will receive one (1) injection which will be administered intravenously at a dose of 2 ml/kg of body weight. If acute toxicity is observed, then reduce the dose to 1 ml/kg for both the control and test articles. A reference for a maximum bolus dose of 2 ml/kg is recommended in Principles and Methods of Toxicology, 2nd Edition, Editor: A. Wallace Hayes, Raven Press, New York, 1989, p. 862. This study dose does not exceed this recommendation.

B. Method

The test and control articles will be administered in an alternating pattern (control rat, test rat, control rat, etc.) with an intravenous injection in one of the lateral tail veins.

1. Properly restrain the rat using a commercial rat restrainer.

2. Position restrainer over a container of very warm water, with the rats tail in the water for several minutes (to increase blood flow).

3. Apply a tourniquet at the base of the tail using a rubber band and hemostats (to tightly clamp rubber band).

4. Re-enter tail into container of water for several minutes.

5. Vigorously rub tail injection area with 70% alcohol before injecting.

6. Insert the 25 gauge needle attached to an appropriately sized syringe (1 ml) into the tail vein and release tourniquet.

7. Inject the control and test articles cautiously, but at a reasonable rate (average = 15-30 seconds).

8. Remove needle/syringe from vein and apply pressure to area with a 2x2 gauze until bleeding stops.

IX. Type/Frequency of Tests

A. Scale Calibration - To be performed on a pre-weighing and post-weighing basis when the rats are weighed, when the weighing boats are weighed, and when the rat organs are weighed on the day of sacrifice.

B. Body Weight Gain - The rats will be weighed Monday through Friday during the one week acclimation period and during the two week study period.

C. Monitoring

1. Physical - The rats will be observed daily for any changes in food or water consumption and for tissue reactions at the site of the injection.

2. Toxicological - The rats will be observed after the injection for signs of acute toxicity as described in Principles and Methods of Toxicology, 2nd Edition, Editor: A. W. Hayes, 1989, p. 180-181.

D. Clinical

1. On the day of the kill (fourteen days after the dosing injection) the following blood samples will be drawn with a heart puncture, after the rat is anesthetized with sodium pentobarbital, for testing:

a. 3 ml EDTA vacutainer tube for CBC with differential (ANTECH Diagnostics Test #951); with the rat's limited total blood volume, only transfer approximately 1-1.5 ml of blood to the EDTA tube and invert tube a minimum of ten (10) times to mix.

b. 4 ml SST (Serum Separator Tube) vacutainer tube for diagnostic Superchem screen (ANTECH Diagnostics Test #951); invert tube five times to mix the clot activator and blood, allow blood to clot for at least 20 minutes, then centrifuge at full speed for 15 minutes.

2. Sort each rat's labeled EDTA tube and labelled Serum Separator Tube with a completed ANTECH Diagnostics Test Requistion form (ANTECH Diagnostics Account Number #31104260-6) into a plastic bag (one per rat); place specimen bags into ANTECH Shipping Box (provided); call FED EX at 1-800-463-3339 for pick-up.

3. Samples will be transported by FED EX to ANTECH Diagnostics (Phone: 1-888-397-8378) for testing.

E. Organ Weights - On the day of the kill (fourteen days after the dosing injection) the following body organs will be examined grossly for abnormalities, collected, and their weights recorded:

1.	Thymus	Small weighing boat
2.	Lungs (both)	Small weighing boat
3.	Heart	Small weighing boat
4.	Spleen	Small weighing boat
5.	Kidneys (both, peel off capsule before weighing)	Small weighing boat
6.	Liver	Medium weighing boat
7.	Testes (both)	Small weighing boat
8.	Brain	Small weighing boat

Note: The weighing boat size is selected to accommodate the total organ and is pre-weighed.

F. Histological Preparation

1. After the specified organs have been weighed, the organs will be placed into a jar containing formalin. Each jar will be pre-labeled with the rat's study number and the date. The organs will be prepared for the fixative process by placing them in the formalin in the following manner:

a. Lungs - with scissors/mid-section slice (from 2 lobes)

b. Heart - whole organ into fixative

c. Spleen - whole organ into fixative

d. Kidneys - with razor blade/butterfly each

e. Liver - with razor blade/mid-section slice (from 2 lobes)

f. Testes - whole organs (both) into fixative

g. Brain - whole organ into fixative (use separate formalin jar)

2. The organs or organ sections will be allowed to fix in the formalin for at least 24 hours; the fixed organ or organ section will then be removed from the formalin and sectioned into properly sized pieces to fit into the histological cassette used during the embedding process.

3. The formalin jars containing the sectioned organs and a Histological Preparation Request Form, will be delivered to the Pathology Department, School of Medicine, SUNY at Buffalo, for processing:

Request Form includes:

a. Dehydration and Embedding -R

b. Sectioning - 5 um

c. Stain - H&E

4. Histology slides will be read by the pathologist, Peter A. Nickerson, A.B., M.A., Ph.D. - Professor and Director of the Pathology Graduate Program, 212 Cary Hall, SUNY at Buffalo. The complete pathology procedure is attached to the end of this protocol.

X. Records

A. "Study 27" Data Notebook will be kept in Farber Hall - Room 118G, SUNY at Buffalo:

1. Inventory of control and test articles received from the sponsor and the animals on which it was administered; paper work for returned control and test articles to the sponsor.

2. Information on rats: Shipment information, initial weights, sex, physical condition.

3. Scale calibration: Scales used for rat weights, organ weights, and weighing boat weights.

4. Rat body weights, with a group mean weight ± Standard Deviation.

5. Daily physical observations of rats.

6. Injection day data: Date, Rat weights, Dose volume, Time, Vial #of article injected, Physical observations (signs of acute toxicity), Post-mortem report of any deaths.

7. Weight of organs at sacrifice: Brain, Thymus, Lungs, Heart, Spleen, Kidneys, Liver, Testes.

8. ANTECH Diagnostics Test Request Form (copy) for the clinical diagnostic tests to be performed on each rat.

9. Histology Request Forms for the tissue specimens submitted for pathology.

10. Hematology test results (Superchem and CBC with differential) on each rat, as performed by ANTECH Diagnostics.

11. Compilation chart of hematology test results.

12. Compilation chart of organ weights with organ/body weight ratios for each rat.

13. Pathology report of histology slides, as prepared by Dr. Peter A. Nickerson.

14. Statistical findings.

B. Histology tissue blocks.

C. Histology slides.

XI. Approval of Protocol

A. By Sponsor: R.E. Counsell 8/21/98
Dr. Raymond Counsell Date

B. By Study Director: Paul Kostyniak 6/2/98
Dr. Paul J. Kostyniak Date

XII. Statistical Methods

Differences in body weights and biochemical parameters will be compared between groups using the t-test.

XIII. Revisions

Any revisions made to this protocol will be attached in the appendices.

XIV. Materials and Equipment

A. Scales

1. AND EK-1200G (Serial #J8025023) - Measures to 0.1 grams, at the Laboratory Animal Facilities, SUNY at Buffalo.

2. Sartorius 1212 MP (Serial #2907085) - Measures to 0.001 grams, from the Toxicology Research Center, SUNY at Buffalo.

B. Centrifuge - Dynac Benchtop Centrifuge #0101 (Serial #22424), from the Toxicology Research Center, SUNY at Buffalo.

C. Syringes - BD syringes 1 ml and 10 ml, Single dose, Sterile, with Luer Lock tip, purchased from the Laboratory Animal Facilities, SUNY at Buffalo.

D. Needles

1. Monoject 25 gauge hypodermic needles X 5/8" long, purchased from the Laboratory Animal Facilities, SUNY at Buffalo.

2. Monoject 20 gauge hypodermic needles x 1" long, purchased from the Laboratory Animal Facilities, SUNY at Buffalo.

Note: Monoject needles are sharper than BD needles

E. Instruments - Large dissection scissors, Small dissection scissors, Scalpel handle #4, Scalpel blades #21, Forceps, Rongeur, Spoon, Single-edged razor blades.

F. Drugs - Sodium Pentobarbital @ 65 mg/ml, manufactured by Veterinary Laboratories, Inc. for the Butler Company, Lot #970789, Expiration Date - 2/00. This will be purchased from the Laboratory Animal Facilities, SUNY at Buffalo, on an ml's as needed basis.

G. ANTECH Diagnostics

1. 3 ml EDTA vacutainer tube (Lavender top), Becton Dickinson #366385, Lot #8B107, Expiration Date: FEB00.

2. 4 ml Serum Separator Tube (SST Vacutainer - Red/Gray top), Becton Dickinson #366514, Lot #8C912, Expiration Date: FEB99.

3. Plastic bags for transporting specimens (a bag for each animals blood tubes).

4. ANTECH Diagnostics Hematology Request Forms with pre-printed Toxicology Research Center Account #31104260-6 and address.

5. FED EX Shipping boxes.

H. Miscellaneous

1. Lab table soaker paper for kill (individual pieces per rat), rolls purchased from Biochemistry Stockroom, Farber Hall - Room 10, SUNY at Buffalo.

2. Glass cutting board for sectioning organs for pathology, Toxicology Research Center, SUNY at Buffalo.

3. Polystyrene weigh boats

   a. Small: 37x10 mm, Laboratory Products Sales (Catalog # D205-1), purchased from Biochemistry Stockroom, Farber Hall - Room 10, SUNY at Buffalo.

   b. Medium: 78x19 mm, Laboratory Products Sales (Catalog # D205-2), purchased from Biochemistry Stockroom, Farber Hall - Room 10, SUNY at Buffalo.

4. Formalin jars - Nalgene, 250 mL (8 oz.) with polypropylene cap, from VWR Scientific Products, Catalog # 16129-378.

5. Formalin - "Z-Fix" prepared by and obtained from Pathology Department, Farber Hall - Room 202C, SUNY at Buffalo (Concentrate from Anatech Ltd, 1020 Harts Lake Road, Battle Creek, Michigan 49015, Phone: 1-800-Anatech).

6. Disposable latex exam gloves, purchased from Biochemistry Stockroom, Farber Hall - Room 10, SUNY at Buffalo.

PATHOLOGY PROCEDURES

The contents of the jar containing tissue from one animal are poured into a sieve. The formalin solution is drained off and collected into a separate container for proper disposal. Technicians in the histology laboratory prepare two processing plastic cassettes with the exact number that matches the number on the jar and the number on the list. The liver, lung, heart, spleen, kidney and testes are sliced with a safety razor blade to a thickness of 2 mm and placed into the cassette. The cassette is then dropped into a running water bath. The brain is sliced transversely to include the basal ganglia in the cerebrum and the cerebellum. The brain tissues are processed in a similar manner by being placed in a cassette and dropped into the water bath.

The tissues are processed by standard procedures for preparation of histological sections: dehydration through several concentrations of alcohols, xylene and paraffin embedding with a 5 micron section fixed on a microscope slide. The slide is then stained with hematoxylin and eosin and cover slipped. The identification number is consistently marked on each slide.

Each slide is microscopically examined:

1. The number of the slide is recorded, the entire section on each slide is surveyed at low power (40 X), for orientation of the section, histological components and any abnormalities visible at this magnification

2. The entire section is viewed with medium power (100 X), and any abnormalities are noted.

3. With high dry power (400 X) the individual histologic components of the section are carefully examined and any abnormality differing from the normal histological appearance is noted and recorded.

After reading all the slides and recording histopathological changes, general comments and comparisons are made. A report on the findings is prepared, signed and presented to the Toxicology Research Center.

Peter A. Nickerson

Peter A. Nickerson, Ph.D.
Professor of Pathology

STUDY 28 – FINAL REPORT

NM-404

Acute Toxicology Study in the Rabbit

The purpose of this study was to evaluate the toxicity in rabbits of phospholipid ether NM-404 (alkyl chain length of 18 carbons), a radioimaging agent for tumors. The control and test articles were formulated by Raymond E. Counsell, Ph.D., Professor of Pharmacology & Medicinal Chemistry, Department of Pharmacology, The University of Michigan Medical School, Ann Arbor, Michigan. The test article was NM-404 in a solution of 2% Tween 20 and sterile water. The control article was only 2% Tween 20 and sterile water. The sponsor of this project was responsible for the specifications of the test and control articles with concern for contaminants that could reasonably be expected to be present and capable of interfering with the purpose of this study. All procedures followed during this study are included in "Study 28 – Protocol" which is attached at the end of this report.

The control and test articles were received from Dr. Counsell on October 29, 1998. At the University of Buffalo, the study test site, four (4) vials of test articles labeled "NM-404 in 2% Tween 20/Sterile Water, MAL-V1-82" and four (4) vials of control articles labeled "Control Vehicle – 2% Tween 20/Sterile Water, MAL-V1-83" were inventoried and stored at room temperature in Farber Hall, Room 111. It was determined after the injections were completed in Study 27 – NM-404 (Acute Rats) that more test and control article would be needed in order to complete the injections to the rabbits. Therefore, on December 4, 1998 an additional vial of test and control article, from the original formulations, were received from Dr. Counsell. All the vials were dated October 16, 1998. The test article of the NM-404 solution was to be administered at approximately 200 times the clinical dose at a concentration of 2 mg/ml and a dose of 4 mg/kg. Each vial was reported to have an approximate volume of 10 ml. A green sticker dot was attached to each test vial to designate the NM-404 solution from the control solution; the control vials were designated with a "C" and then each set of control and test vials were numbered #1 to #5. Control vial #2C and test vial (with a green dot) #2 were injected into the rabbits on January 13, 1999 (Day 0 of the study). Control and test vials #1 were used exclusively for the rats and were not used for any of the rabbits. The control and test rabbits were injected intravenously in the lateral ear vein at a dose of 2 ml/kg.

On January 5, 1999 sixteen (16) New Zealand White rabbits were received from HRP, Inc. (Covance), Denver, Pennsylvania. The rabbits were all males, all born on November 7, 1998, and all appeared healthy. They were housed at the Laboratory Animal Facility, CFS Addition – Room 122 D. The rabbits were weighed upon arrival and their weights ranged from 1.48 kilograms to 1.68 kilograms. Two groups of eight (8) rabbits per group, controls and tests, were established with a mean weight of each group of 1.55 kilograms and 1.60 kilograms, respectively. The rabbits were housed one (1) animal per cage and given water ad lib. The food was at first restricted and then increased daily over a 5 day period until they were fed a maximum amount of 125 grams. This occurred during their 7 day quarantine period. Each rabbit was ear tagged with a metal tag imprinted with an individual number by the Laboratory Animal Facilities. For the purpose of this study, a study number was written on the ear of each rabbit that was a unique number of '1' to '16', numerically. The control rabbits were numbered '1' to '8' and the test rabbits were numbered '9' to '16". The unique numbers were also applied to each cage indicating which rabbit was housed within.

On January 13, 1999 (Day 0) the eight (8) control and eight (8) test rabbits were restrained, their ears cleaned with alcohol, and injected intravenously in the lateral ear vein at 2 ml/kg of body weight using a 25 gauge needle and a 5 ml syringe. The injections were given by alternating a rabbit from the control group with a rabbit from the test group, with the injections given over a 1 minute to 5 minute interval. Most of the injection times averaged between 1 – 2 minutes. The injections were administered in the left ears of the rabbits, except in test rabbit #28-09 when both ears received a portion of the total dose. Control rabbits #28-03, #28-04, #28-05, #28-06 received the injection in 2 sites; also test rabbit #28-09 and #28-11 received the injection in 2 sites. This occurred because the rabbit moved during the initial injection procedure. The injection on control rabbit #28-01 was given at 9:32 A.M. and the last injection on test rabbit #28-16 was given at 11:05 A.M. No adverse reactions were observed at the time of the injection or noted after the injections were completed. The rabbits were observed for signs of acute toxicity as described in Principles and Methods of Toxicology, 2nd Edition, Editor: A.W. Hayes, 1989, p. 180-181. The rabbits were observed closely until 1:45 P.M., and throughout the afternoon. No unusual behavior was noted in any of the rabbits during this time or during the remainder of the study.

The ear injection sites were observed daily. On Day 1 the left ear of control rabbit #28-01 was slightly red around the injection site; the left ear of control rabbit #28-03 was reddish purple and warm for almost the entire length of the ear and past the medial artery; the left ear of control rabbit #28-05 was bruised along the vein and below the injection site; and the left ear of test rabbit #28-10 was red around the injection site. On Day 2 the left ear of control rabbit #28-01 was improved – the redness was gone but the vein was slightly bruised at the injection site; the left ear of control rabbit #28-03 was improved – the area was less discolored and no longer warm but there was bruising along the vein; the left ear of control rabbit #28-05 did not show any improvement with the bruising still present; and the left ear of test rabbit #28-10 was normal. On Day 5 the left ears of control rabbits #28-01 and #28-03 were normal. The left ear of control rabbit #28-05 was improved but still had a 1 ½ inch thickened and discolored area along the

vein. Day 6 showed the left ear of control rabbit #28-05 to be improving with less discoloration and less thickening along the vein. The left ear of control rabbit #28-05 continued to improve until Day 12 when the ear and vein were normal.

The rabbits were weighed five (5) times a week (Monday through Friday) and their weights, recorded in kilograms, appear in Table 1. The mean weights of the control group and the test group also appear in Table 1 and these values are compared in Graph 1. Some rabbits did show a weight loss for a day or two and this could be attributed to the time of feeding or the time of normal bodily functions. Weight gain appears to be normal between the two groups.

The rabbits were anesthetized with sodium pentobarbital (65 mg/ml, Lot #970789, Expiration Date: 2/00 and Lot #980410, Expiration Date: 6/00) administered intravenously in the lateral ear vein on January 27, 1999. A heart puncture was then performed using a 21 gauge vacutainer needle and cuff to collect the blood samples for hematology testing. The rabbits were then overdosed with sodium pentobarbital until death occurred. The brain, heart, lungs, thymus, spleen, kidneys (both), liver, and testes (both) were collected, examined grossly, weighed, and sectioned for pathology. The organ weights and the organ to final body weight ratios data appears in Table 2. The organ/body weight ratios were compared using a Students t-test and the only significant difference was found when comparing the thymus/body weight ratio of the control and test rabbits. The tissue samples (except thymus) were placed in jars of 'Z-Fix' fixative. The following week, the organs were examined by the pathologist, Dr. Peter Nickerson, and cut into a representative section for processing for histopathological examination. Dr. Nickerson reports that "no gross lesions were noted in any of the groups receiving either the test material or in the control group". The collected organs were processed by the Department of Pathology, SUNY at Buffalo, School of Medicine. The histological preparations were examined by Peter A. Nickerson, Ph.D., Professor of Pathology, SUNY at Buffalo. The report from Dr. Nickerson lists his findings for control rabbit #28-01 to #28-08 and for test rabbit #28-09 to #28-16, with a description of each organ. Dr. Nickerson concludes that "by light microscopic examination, there are no changes in the histopathology of the organs examined that can be attributed to administration of the test material. Brain, kidney (casts observed in both groups are within normal limits), liver, spleen, testis and heart were within normal limits in the control and in the test group. In rabbits #6 and #7 for controls and #10 in the test material groups there are a few granulomas in the area of the triad. Also, in the control group, rabbit #6, a granuloma is observed in the myocardial septum. These findings of granulomas are known to occur in rabbits and are not attributable to the test material". The complete histological report prepared by Dr. Nickerson is attached.

Clinical blood chemistries (Superchem) and a CBC (Complete Blood Count with differential) were performed by Antech Diagnostics, Memphis, Tennessee on January 28, 1999 (Day 14). The results of the Superchem screen and CBC appear in Table 3 (Page 1: Control Rabbits #1- #8; Page 2: Test Rabbits #9 - #16; Page 3: Mean Data with Standard Deviation for both groups). The values were checked for any obvious low or high values that were below or above the reference range provided by Antech

Diagnostics. Whenever any value was outside of this reference range the groups were compared using a Students t-test at a p value of less than 0.05. T-tests were performed for the following blood tests: Calcium, Phosphorus, Sodium, Potassium, Triglycerides, AST (SGOT), ALT (SGPT), Alkaline phosphatase, Protein, Globulin, A/G ratio, Urea Nitrogen, Glucose, WBC, Hemoglobin, Hematocrit, and MCV. A significant difference was found with the A/G ratios when these values of the control and test rabbits were compared. The A/G ratio values for both the control and test rabbits were higher than the given reference range of 0.9 to 1.7. The control rabbit values ranged from 2.0 to 3.0 (Mean $\pm$ STD = 2.6 $\pm$ 0.3); the test rabbit values ranged from 2.6 to 3.3 (Mean $\pm$ STD = 2.9 $\pm$ 0.2). The WBC values also showed a significant difference between the control and test rabbits. The given reference range was 4.0 to 10.0 thds/cmm. Four (4) control rabbits had lower WBC values, with three (3) control rabbits within the normal reference range; all eight (8) test rabbits were reported to have lower WBC values. The control rabbit Mean $\pm$ STD was 3.4 $\pm$ 1.1 and the test rabbit Mean $\pm$ STD was 1.7 $\pm$ 0.9. No other significant differences were found. The platelet estimation was similar between the control and test rabbits (Control rabbits: adequate in 7 rabbits, decreased in 1 rabbit; Test rabbits: adequate in 7 rabbits, decreased in 1 rabbit). Platelets were found to be clumped in only test rabbit #28-11. Slight anisocytosis was reported in control rabbit #28-02 and #28-08 and test rabbit #28-10. Moderate anisocytosis was only reported in control rabbit #28-04. Polychromasia was only reported in test rabbit #28-15.

External factors which might effect the study outcome appear to have been very limited in this study. Daily observations of the rabbits were also made by the personnel of the Laboratory Animal Facilities, SUNY at Buffalo.

At the conclusion of this study, all data notebooks with reports, histology tissue blocks, and stained slides are stored in Farber Hall, Room 118G at SUNY at Buffalo. All materials are labeled as "Study 28".

During the course of this study, an attempt was made to follow the Good Laboratory Practice (GLP) Regulations as outlined in the Federal Register of December 22, 1978 and September 4, 1987, Final Rule (21 CFR Part 58). Technical procedures are described in the "Study 28 – Protocol NM-404 Acute Toxicology Study in the Rabbit" which is attached at the end of this report.

At the State University of New York at Buffalo, the Study Technician was Ellen M. Schopp under the supervision of the Project Director, Paul J. Kostyniak, Ph.D. A special thanks to Marian M. Pazik for his assistance and cooperation during this study and for his technical support in the compiling of the study data and to Joseph A. Syracuse for his support on the final day. The Quality Assurance Officer who reviewed this report was Hebe B. Greizerstein, Ph.D.

Reviewed and Approved by:

Dr. Paul J. Kostyniak
Study Director

3-24-99
Date

Ellen M. Schopp
Study Technician

3/23/99
Date

Dr. Hebe B. Greizerstein
Quality Assurance Officer

3/23/99
Date

STUDY # 28

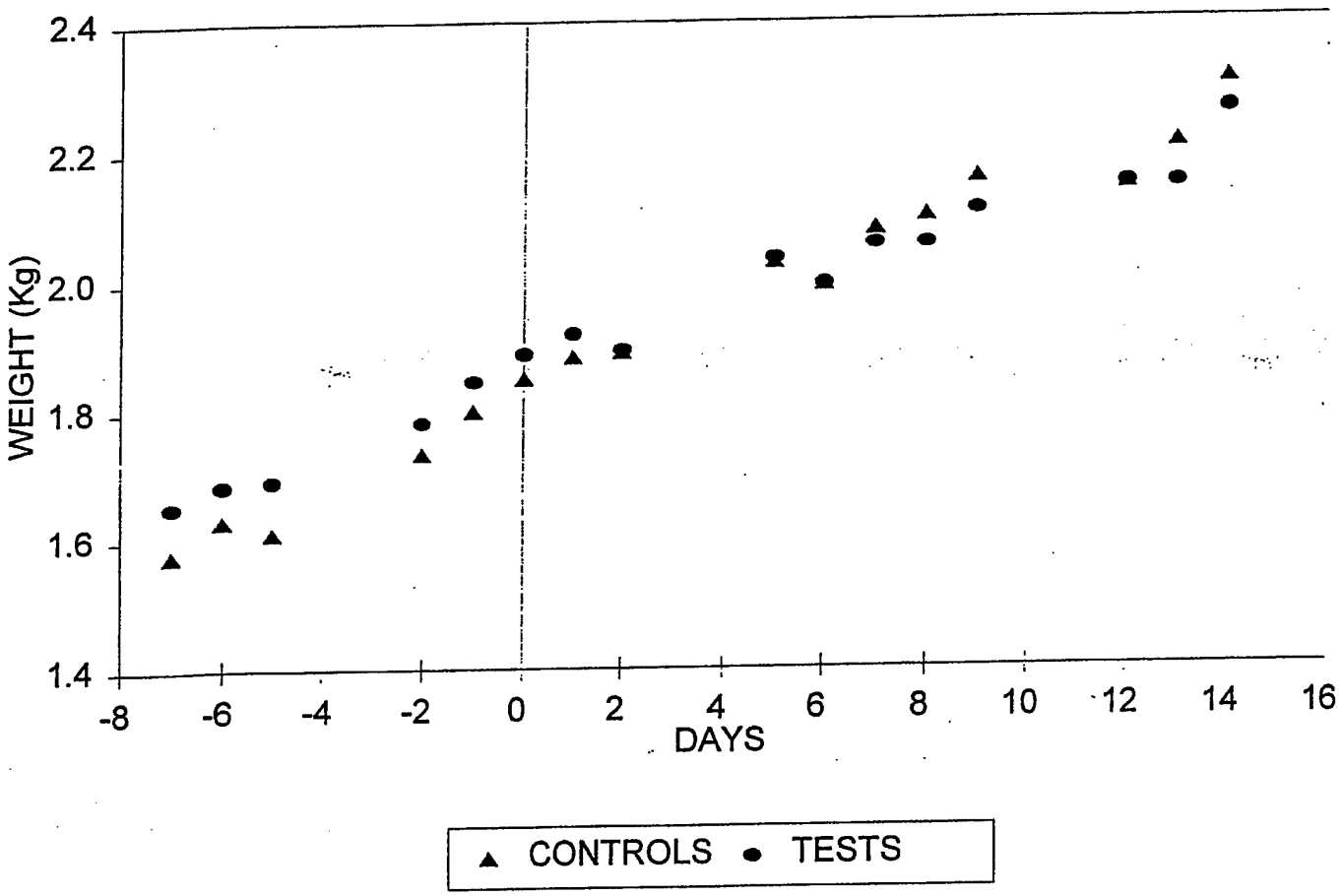
DESCRIPTION : NM-404: Rabbits / Acute

RABBIT WEIGHTS (Kilograms)

DAY	-9	-8	-7	-6	-5	-2	-1	0	1	2	5	6	7	8	9	12	13	14
CONTROLS																		
28-01			1.480	1.390	1.390	1.590	1.620	1.680	1.675	1.680	1.795	1.775	1.885	1.900	1.890	1.910	1.960	2.015
28-02			1.640	1.705	1.655	1.760	1.835	1.935	1.955	1.965	1.970	1.910	1.980	2.060	2.165	2.135	2.230	2.255
28-03			1.630	1.665	1.620	1.805	1.930	1.960	2.040	1.990	2.165	2.205	2.250	2.255	2.365	2.270	2.340	2.500
28-04			1.665	1.690	1.680	1.795	1.800	1.910	1.915	1.895	2.040	1.985	2.065	2.100	2.095	2.155	2.195	2.260
28-05			1.495	1.650	1.640	1.700	1.705	1.650	1.675	1.760	1.925	1.910	1.910	1.930	1.995	2.040	2.055	2.170
28-06			1.495	1.615	1.655	1.755	1.795	1.890	1.925	1.925	2.145	2.065	2.200	2.225	2.245	2.225	2.355	2.415
28-07			1.635	1.735	1.620	1.745	1.930	1.920	2.005	2.025	2.115	2.060	2.225	2.205	2.300	2.225	2.250	2.445
28-08			1.585	1.590	1.625	1.725	1.775	1.855	1.870	1.885	2.075	2.045	2.120	2.120	2.205	2.205	2.285	2.400
AVG			1.58	1.63	1.61	1.73	1.80	1.85	1.88	1.89	2.03	1.99	2.08	2.10	2.16	2.15	2.21	2.31
STD			0.07	0.10	0.09	0.06	0.10	0.11	0.13	0.11	0.12	0.12	0.13	0.12	0.15	0.11	0.13	0.15
MEAN+STD			1.65	1.73	1.70	1.80	1.90	1.96	2.01	2.00	2.15	2.12	2.21	2.22	2.31	2.26	2.34	2.46
MEAN-STD			1.51	1.53	1.52	1.67	1.70	1.74	1.75	1.78	1.91	1.87	1.95	1.98	2.01	2.03	2.08	2.16
TESTS																		
28-09			1.765	1.690	1.685	1.750	1.855	1.925	1.915	1.875	2.070	2.025	2.015	1.995	2.050	2.110	2.065	2.165
28-10			1.535	1.560	1.610	1.740	1.850	1.840	1.875	1.845	1.965	1.875	2.010	2.035	2.100	2.070	2.015	2.190
28-11			1.540	1.775	1.730	1.810	1.845	1.895	2.005	1.960	2.095	2.090	2.150	2.130	2.160	2.260	2.245	2.365
28-12			1.650	1.610	1.710	1.860	1.890	1.920	1.995	2.035	2.060	2.040	2.120	2.040	2.060	2.120	2.155	2.255
28-13			1.570	1.575	1.560	1.725	1.810	1.820	1.840	1.805	1.950	1.960	2.010	2.065	2.095	2.115	2.100	2.120
28-14			1.710	1.805	1.760	1.850	1.890	1.950	1.965	1.935	2.095	2.070	2.160	2.120	2.210	2.235	2.320	2.410
28-15			1.770	1.745	1.705	1.745	1.800	1.860	1.870	1.830	1.985	1.930	1.970	2.010	2.095	2.105	2.115	2.220
28-16			1.685	1.720	1.775	1.770	1.815	1.890	1.890	1.860	2.060	1.970	2.010	2.055	2.080	2.155	2.150	2.355
AVG			1.65	1.69	1.69	1.78	1.84	1.89	1.92	1.89	2.04	2.00	2.06	2.06	2.11	2.15	2.15	2.26
STD			0.09	0.09	0.07	0.05	0.03	0.04	0.06	0.07	0.06	0.07	0.07	0.04	0.05	0.06	0.09	0.10
MEAN+STD			1.74	1.77	1.76	1.83	1.88	1.93	1.98	1.97	2.09	2.06	2.13	2.10	2.16	2.21	2.24	2.36
MEAN-STD			1.56	1.60	1.62	1.73	1.81	1.85	1.86	1.82	1.98	1.93	1.99	2.01	2.06	2.08	2.05	2.16

Table 1

STUDY 28: NM-404
Rabbits - Acute



STUDY # 28

DESCRIPTION: NM-404: Rabbits / Acute

ORGAN WEIGHTS AND ORGAN/BODY WEIGHT RATIOS

CONTROLS	Final Body Weight (kg)	Final Body Weight (kg)	Brain (g)	Brain/BW Ratio	Testes (g)	Testes/BW Ratio	Liver (g)	Liver/BW Ratio	Kidneys (g)	Kidney/BW Ratio
28-01	2.015	2.015	8.24	4.089	0.85	0.422	68.14	33.816	12.24	6.074
28-02	2.255	2.255	8.55	3.792	1.08	0.479	56.41	25.016	13.34	5.916
28-03	2.500	2.500	8.30	3.320	1.00	0.400	76.18	30.472	12.38	4.952
28-04	2.260	2.260	8.33	3.686	1.25	0.553	66.92	29.611	11.92	5.274
28-05	2.170	2.170	8.22	3.788	1.00	0.461	68.39	31.516	13.43	6.189
28-06	2.415	2.415	8.67	3.590	1.36	0.563	63.22	26.178	13.19	5.462
28-07	2.445	2.445	8.58	3.509	1.58	0.646	77.28	31.607	13.73	5.616
28-08	2.400	2.400	8.11	3.379	0.66	0.275	85.74	35.725	13.92	5.800
Mean	2.308	2.308	8.375	3.644	1.098	0.475	70.285	30.493	13.019	5.660
STD	0.152	0.152	0.187	0.234	0.273	0.107	8.548	3.356	0.693	0.392
Mean + STD	2.460	2.460	8.56	3.88	1.37	0.58	78.83	33.85	13.71	6.05
Mean - STD	2.155	2.155	8.19	3.41	0.82	0.37	61.74	27.14	12.33	5.27
TESTS										
28-09	2.165	2.165	8.13	3.755	1.72	0.794	65.25	30.139	13.41	6.194
28-10	2.190	2.190	8.38	3.826	1.12	0.511	65.22	29.781	11.29	5.155
28-11	2.365	2.365	8.62	3.645	1.04	0.440	74.72	31.594	13.24	5.598
28-12	2.255	2.255	8.12	3.601	1.18	0.523	69.70	30.909	11.56	5.126
28-13	2.120	2.120	8.64	4.075	1.26	0.594	62.47	29.467	12.40	5.849
28-14	2.410	2.410	8.17	3.390	1.32	0.548	77.10	31.992	14.65	6.079
28-15	2.220	2.220	7.53	3.392	1.07	0.482	92.09	41.482	14.96	6.739
28-16	2.355	2.355	8.11	3.444	2.15	0.913	77.72	33.002	11.17	4.743
Mean	2.260	2.260	8.213	3.641	1.358	0.601	73.034	32.296	12.835	5.685
STD	0.099	0.099	0.330	0.224	0.361	0.155	9.006	3.646	1.382	0.616
Mean + STD	2.359	2.359	8.54	3.87	1.72	0.76	82.04	35.94	14.22	6.30
Mean - STD	2.161	2.161	7.88	3.42	1.00	0.45	64.03	28.65	11.45	5.07

Table 2

STUDY # 28

DESCRIPTION: NM-404: Rabbits / Acute

ORGAN WEIGHTS AND ORGAN/BODY WEIGHT RATIOS

CONTROLS	Final Body Weight (kg)	Spleen (g)	Spleen/BW Ratio	Heart (g)	Heart/BW Ratio	Lungs (g)	Lung/BW Ratio	Thymus (g)	Thymus/BW Ratio
28-01	2.015	1.08	0.536	8.91	4.422	9.00	4.467	3.45	1.712
28-02	2.255	1.47	0.652	4.19	1.858	8.60	3.814	3.23	1.432
28-03	2.500	0.89	0.356	9.08	3.632	8.51	3.404	4.07	1.628
28-04	2.260	1.37	0.606	4.78	2.115	8.61	3.810	4.05	1.792
28-05	2.170	1.34	0.618	4.63	2.134	8.40	3.871	3.74	1.724
28-06	2.415	1.26	0.522	4.53	1.876	8.10	3.354	3.88	1.607
28-07	2.445	1.06	0.434	4.74	1.939	8.63	3.530	4.31	1.763
28-08	2.400	0.93	0.388	5.44	2.267	8.52	3.550	3.83	1.596
Mean	2.308	1.175	0.514	3.151	2.530	8.546	3.725	3.820	1.657
STD	0.152	0.201	0.104	2.954	0.896	0.235	0.335	0.326	0.109
Mean + STD	2.460	1.38	0.62	6.10	3.43	8.78	4.06	4.15	1.77
Mean - STD	2.155	0.97	0.41	0.20	1.63	8.31	3.39	3.49	1.55
TESTS									
28-09	2.165	0.64	0.296	5.83	2.693	8.20	3.788	3.75	1.732
28-10	2.190	1.11	0.507	4.51	2.059	7.86	3.589	4.10	1.872
28-11	2.365	0.86	0.364	4.96	2.097	10.13	4.283	4.00	1.691
28-12	2.255	0.99	0.439	4.47	1.982	8.02	3.557	3.34	1.481
28-13	2.120	1.24	0.585	4.50	2.123	8.50	4.009	3.90	1.840
28-14	2.410	0.87	0.361	4.91	2.037	8.41	3.490	4.80	1.992
28-15	2.220	0.76	0.342	5.99	2.698	8.66	3.901	4.89	2.203
28-16	2.355	1.09	0.463	6.55	2.781	8.40	3.567	4.58	1.945
Mean	2.260	0.945	0.420	5.215	2.309	8.523	3.773	4.170	1.844
STD	0.099	0.186	0.090	0.749	0.325	0.654	0.259	0.507	0.203
Mean + STD	2.359	1.13	0.51	5.96	2.63	9.18	4.03	4.68	2.05
Mean - STD	2.161	0.76	0.33	4.47	1.98	7.87	3.51	3.66	1.64

STUDY # 28

TITLE: NM-404: Rabbits / Acute

Blood Chemistry & CBC Results -

DAY # 14

ANIMAL # / BLOOD TEST	TESTS							
	28-09	28-10	28-11	28-12	28-13	28-14	28-15	28-16
Calcium mg/dL	14.3	15.0	14.4	15.7	13.9	15.0	13.9	14.2
Phosphorus mg/dL	8.5	5.6	6.1	4.6	5.7	4.0	5.6	4.2
Sodium mEq/L	144	145	142	141	140	140	143	141
Potassium mEq/L	6.6	6.2	7.0	7.6	6.0	6.5	6.6	6.4
Chloride mEq/L	99	106	104	103	103	105	106	103
Cholesterol mg/dL	26	37	41	41	31	38	31	27
Triglycerides mg/dL	34	52	63	77	48	87	97	106
AST (SGOT) U/L	171	46	103	40	66	36	27	25
Bilirubin, Total mg/dL	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1
GGTP U/L	2	2	2	2	2	2	2	2
ALT (SGPT) IU/L	54	67	65	86	70	85	36	40
Alkal. Phosphatase U/L	125	151	125	112	139	106	100	59
Protein, Total g/dL	5.5	5.5	5.3	5.7	5.4	5.1	5.2	5.2
Globulin g/dL	1.4	1.5	1.3	1.6	1.4	1.3	1.2	1.3
Albumin g/dL	4.1	4.0	4.0	4.1	4.0	3.8	4.0	3.9
A/G Ratio	2.9	2.7	3.1	2.6	2.9	2.9	3.3	3.0
Urea Nitrogen mg/dL	16	22	20	28	26	26	26	24
Creatinine mg/dL	1.3	1.1	1.2	1.2	1.0	1.0	1.0	1.1
BUN/Creatinine Ratio	12	20	17	23	26	26	26	22
Glucose mg/dL	166	133	139	154	126	163	141	121
Amylase U/L	255	284	228	330	264	306	274	326
Lipase U/L	269	319	300	499	615	389	356	445
CPK U/L	3999	806	2596	931	2783	1313	917	1251
Magnesium mEq/L	2.4	2.3	2.8	2.9	2.5	2.3	2.3	2.6
Osmolality, Calc'd mosm/	295	296	292	295	288	291	295	289
WBC thds/cmm	2.0	2.1	1.1	3.2	2.0	2.5	0.4	0.5
RBC mill/cmm	6.99	5.40	5.61	5.42	5.91	5.60	5.71	5.68
Hemoglobin g/dL	13.6	11.4	11.2	11.8	12.3	11.3	11.4	11.7
Hematocrit %	42.8	35.4	34.6	36.7	37.6	35.3	35.8	35.8
MCV	61	66	62	68	64	63	63	63
MCH	19.5	21.1	20.0	21.8	20.8	20.2	20.0	20.6
MCHC	31.8	32.2	32.4	32.2	32.7	32.0	31.8	32.7
Polys %	42	28	62	46	72	58	35	43
Bands %	0	0	0	0	0	0	0	0
Lymphocytes %	40	72	38	34	18	32	52	37
Monocytes %	18	0	0	17	10	10	8	18
Eosinophils %	0	0	0	3	0	0	3	2
Basophils %	0	0	0	0	0	0	2	0
Platelet Estimation	Adequat	Adequat	Decreas	Adequat	Adequat	Adequat	Adequat	Adequat
Platelet Comments			Clumps					
Anisocytosis		Slight						
Polychromasia							Slight	
Other Comments								

STUDY # 28

TITLE: NM-404: Rabbits / Acute

Blood Chemistry & CBC Results -

DAY # 14

ANIMAL # / BLOOD TEST	MEAN DATA CONTROLS MEAN	CONTROLS STD	TESTS MEAN	TESTS STD	* Significant Difference
Calcium mg/dL	14.5	1.1	14.6	0.6	
Phosphorus mg/dL	5.5	1.1	5.5	1.3	
Sodium mEq/L	140.9	1.2	142.0	1.7	
Potassium mEq/L	6.0	0.7	6.6	0.5	
Chloride mEq/L	103.1	1.4	103.6	2.1	
Cholesterol mg/dL	40.9	12.1	34.0	5.6	
Triglycerides mg/dL	80.5	43.0	70.5	23.8	
AST (SGOT) U/L	38.6	15.7	64.3	46.8	
Bilirubin, Total mg/dL	0.1	0.0	0.1	0.0	
GGTP U/L	2.5	1.3	2.0	0.0	
ALT (SGPT) IU/L	46.1	12.3	62.9	17.4	
Alkal. Phosphatase U/L	111.5	24.4	114.6	26.3	
Protein, Total g/dL	5.4	0.2	5.4	0.2	
Globulin g/dL	1.5	0.2	1.4	0.1	
Albumin g/dL	3.9	0.2	4.0	0.1	
A/G Ratio	2.6	0.3	2.9	0.2	★
Urea Nitrogen mg/dL	22.8	6.4	23.5	3.7	
Creatinine mg/dL	1.1	0.1	1.1	0.1	
BUN/Creatinine Ratio	21.6	6.8	21.5	4.7	
Glucose mg/dL	141.9	15.5	142.9	15.6	
Amylase U/L	267.9	55.5	283.4	33.2	
Lipase U/L	470.8	152.8	399.0	108.3	
CPK U/L	893.8	344.6	1824.5	1089.3	
Magnesium mEq/L	2.5	0.2	2.5	0.2	
Osmolality, Calc'd mosm/	289.1	4.1	292.6	2.9	
WBC thds/cmm	3.4	1.1	1.7	0.9	★
RBC mill/cmm	5.7	0.4	5.8	0.5	
Hemoglobin g/dL	11.8	0.6	11.8	0.7	
Hematocrit %	36.8	2.1	36.8	2.4	
MCV	65.1	2.0	63.8	2.1	
MCH	20.9	0.7	20.5	0.7	
MCHC	32.1	0.6	32.2	0.3	
Polys %	57.1	15.8	48.3	13.7	
Bands %	0.0	0.0	0.0	0.0	
Lymphocytes %	34.0	17.3	40.4	14.8	
Monocytes %	6.3	5.2	10.1	6.9	
Eosinophils %	1.9	2.4	1.0	1.3	
Basophils %	0.6	0.9	0.3	0.7	
Platelet Estimation					
Platelet Comments					
Anisocytosis					
Polychromasia					
Other Comments					

STUDY 28

Gross Examination:

Organs were examined grossly at the time of removal and after fixation, before a representative section was cut for processing for histopathological examination. No gross lesions were noted in any of the groups receiving either the test material or in the control group.

Histopathological Examination of Organs:

Rabbits receiving the Test Material.

Rabbit #9

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp has normal structure.

Testis: The testis is immature and there are no spermatozoa in the seminiferous tubules.

Rabbit #10

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are

normal; there is no inflammation or other indication of infection.

Heart: The myocardium, endocardium and coronary vessels are normal.

Liver: The lobule structure and the cellular structure of the parenchymal cells are normal; there are no vacuoles within the parenchymal cells of the liver. In several triads there is evidence of macrophages in the interstitial space around the bile duct, hepatic artery and the portal vein.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. A few casts are observed in the tubular system.

Spleen: White and red pulps have normal structure.

Testis: The testis is immature and there are no spermatozoa in the seminiferous tubules.

Rabbit #11

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp has normal structure.

Testis: The testis is immature and there are no spermatozoa in the seminiferous tubules.

Rabbit #12

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp has normal structure.

Testis: The testis is immature and there are no spermatozoa in the seminiferous tubules.

Rabbit #13

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp has normal structure.

Testis: The testis is immature and there are no spermatozoa in the seminiferous tubules.

Rabbit #14

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp has normal structure.

Testis: The testis is immature and there are no spermatozoa in the seminiferous tubules.

Rabbit #15

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp has normal structure.

Testis: The testis is immature and there are no spermatozoa in the seminiferous tubules.

Rabbit #16

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp has normal structure.

Testis: The testis is immature and there are no spermatozoa in the seminiferous tubules.

Control Group:

Rabbit #1

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are

normal; there is no inflammation or other indication of infection.

Liver: The lobule structure and the cellular structure of the parenchymal cells are normal; there is no inflammation or vacuoles within the parenchymal cells of the liver.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. In a few tubules, there are a few eosinophilic casts.

Spleen: White and red pulp have normal structure.

Testis: The testis is immature and there are no spermatozoa in the seminiferous tubules.

Rabbit #2

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection.

Liver: The lobule structure and the cellular structure of the parenchymal cells are normal; there are no vacuoles within the parenchymal cells of the liver.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several focal areas in the renal medulla contain tubules that exhibit eosinophilic casts.

Spleen: White and red pulp have normal structure.

Testis: The testis is immature and there are no spermatozoa in the seminiferous tubules.

Rabbit #3

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection.

Liver: The lobule structure and the cellular structure of the parenchymal cells are normal; there is no inflammation or vacuoles within the parenchymal cells of the liver.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Eosinophilic casts are observed in some medullary tubule cells.

Spleen: White and red pulp have normal structure.

Testis: The testis is immature and there are no spermatozoa in the seminiferous tubules.

Rabbit #4

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection.

Liver: The lobule structure and the cellular structure of the parenchymal cells are normal; there is no inflammation or vacuoles within the parenchymal cells of the liver.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Eosinopilic casts are observed in some medullary tubules.

Spleen: White and red pulp have normal structure.

Testis: The testis is immature and there are no spermatozoa in the seminiferous tubules.

Rabbit #5

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection.

Liver: The lobule structure and the cellular structure of the parenchymal cells are normal; there are no vacuoles within the parenchymal cells of the liver.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. A few eosinophilic casts are seen in the medullary tubules.

Spleen: White and red pulp have normal structure.

Testis: The testis is immature and there are no spermatozoa in the seminiferous tubules.

Rabbit #6

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection.

Liver: The lobule structure and the cellular structure of the parenchymal cells are normal; there are no vacuoles within the parenchymal cells of the liver. In a few of the triads there are macrophages in the interstitium among the bile ducts, hepatic artery and portal vein.

Heart: The endocardium and coronary vessels are normal. In the myocardial septum, one focal area with inflammatory cells (neutrophils and macrophages) extends to the endocardium and resembles a granuloma.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. A few eosinophilic casts are observed in focal medullary tubules.

Spleen: White and red pulp have normal structure.

Testis: The testis is immature and there are no spermatozoa in the seminiferous tubules.

Rabbit #7

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection.

Liver: The lobule structure and the cellular structure of the parenchymal cells are normal; there are no vacuoles within the

parenchymal cells of the liver. Several triads have a small collection of macrophages and are interpreted as a granuloma.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Some tubules with eosinophilic casts are observed.

Spleen: White and red pulp have normal structure.

Testis: The testis is immature and there are no spermatozoa in the seminiferous tubules.

Rabbit #8

Brain: The cerebrum and cerebellum have normal structure; there is no edema, inflammation or infarcts.

Lung: The airways and distal structure of the lung (alveoli) are normal; there is no inflammation or other indication of infection. There is no pulmonary edema.

Liver: The lobule structure is normal.

Heart: The myocardium, endocardium and coronary vessels are normal.

Kidney: The cortex, medulla and urinary space exhibit a normal histology. Several casts are present in the renal tubular system.

Spleen: White and red pulp has normal structure.

Testis: The testis is immature and there are no spermatozoa in the seminiferous tubules.

Interpretation and comments:

By light microscopic examination, there are no changes in the histopathology of the organs examined that can be attributed to administration of the test material.

Brain, kidney (casts observed in both groups are within normal limits), liver, spleen, testis and heart were within normal limits in the control and in the test group.

In rabbits #6 and #7 for controls and #10 in the test material groups there are a few granulomas in the area of the triad. Also, in the control group, rabbit #6, a granuloma is observed in the myocardial septum. These findings of granulomas are known to occur in rabbits and are not attributable to the test material.

Peter A. Nickerson

Peter A. Nickerson, Ph.D.
Professor of Pathology

3/22/99
Date

STUDY 28 - PROTOCOL

NM-404

ACUTE TOXICOLOGY STUDY

IN THE RABBIT

I. Description

The purpose of this study is to evaluate the toxicity in rabbits of phospholipid ether NM-404 (alkyl chain length of 18 carbons), a radioimaging agent for tumors.

II. Control/Test Articles

The formulation of the control and test articles will be performed by the sponsor. The control and test articles received from the sponsor for this study will be stored at room temperature in Farber Hall - Room 111, SUNY at Buffalo, New York. At termination of the study, the remaining control and test articles will be returned to the sponsor for QC and integrity testing.

Control Article: The control solution will be 2% Tween 20 and sterile water.

Test Article: The NM-404 solution will be NM-404 in a solution of 2% Tween 20 and sterile water. The test article of NM-404 solution will be approximately 200 times the clinical dose with a concentration of 2 mg/ml.

III. Sponsor/Testing Facility

Sponsor: Raymond E. Counsell, Ph.D.
Professor of Pharmacology & Medicinal Chemistry
Department of Pharmacology
1301 Medical Science Research Building
The University of Michigan Medical School
Ann Arbor, Michigan 48109-0632
Office: 313-764-8165
FAX: 313-763-4450

Project Director: Paul J. Kostyniak, Ph.D.
Director, Toxicology Research Center
Farber Hall - Room 111
SUNY at Buffalo
Office: 716-829-2125
FAX: 716-829-2806

Testing Facility: SUNY at Buffalo
Laboratory Animal Facilities
CFS Addition
Main Street Campus
Buffalo, New York 14214-3000

Laboratory Animal Facilities
Director: Thomas Martin, BVSC DipVetPath PhD MBA MACVSc DiplACLAM
Laboratory Animal Facilities
116 CFS Addition
SUNY at Buffalo
Office: 716-829-2919
FAX: 716-829-3249

The Institutional Animal Care and Use Committee (IACUC) at the University of Buffalo has approved this study with the animal use project number of PMY22074N.

IV. Test System

Rabbit Supplier: HRP, Inc. (Covance)
P.O. Box 7200
Denver, Pennsylvania 17517
Phone: 1-800-345-4114
FAX: 717-336-5344

Rabbit Description: New Zealand White
Specific Pathogen Free (S.P.F.)
Male
3.5-4.0 lbs.
Quantity - 16 animals

The rabbits will be housed at the State University of New York at Buffalo, Laboratory Animal Facilities, CFS Addition, Room 122 D.

V. Identification of Test System

Each rabbit will be given an individual animal number by the Laboratory Animal Facilities. This number is stamped into a metal tag that is applied to the ear of each rabbit and identifies it from any other rabbit in the facility. Each rabbit will also have a two-part number starting with 28-(Study #), followed by a 'unique' number of '01' to '16' (numerical). The unique number will be applied to the hairless (inner) side of the right ear with a Sanford Sharpie Fine Point Permanent Marker and re-applied when the number begins to wear off. Each rabbit, housed one (1) animal per cage, will have a cage card with the unique number indicated, applied with the permanent marker, and reflective of the rabbit housed within.

When referring to any rabbit during this study the 'unique' number of '01' to '16' will be used.

IV. Experimental Design

A. The sixteen (16) rabbits are randomly divided into two (2) groups having approximately the same mean weight:

1. Control Group: Eight (8) rabbits to receive the control article of 2% Tween 20

2. Test Group: Eight (8) rabbits to receive the test article of NM-404 in 2% Tween 20

B. The rabbits are weighed and their weights recorded in kilograms (kg), during the one week quarantine period

and during two week study period, Monday through Friday, and more often if problems with weight gain occur.

C. The rabbits will be observed for any unusual behavior or change in food and water intake for the duration of the study.

D. The rabbits will be injected intravenously in the lateral ear vein using sterile techniques, and an appropriately sized syringe with a 25 gauge needle (see Section VIII, Part B).

E. Initially, one (1) control and one (1) test rabbit will be injected at 2 ml/kg with the appropriate dosing solution:

1. These rabbits will be observed for any signs of toxicity, respiratory distress, change in motor activity, seizures, etc. (see Section IX, Part C).

   a. If any deaths are observed, the remaining rabbits will be injected at 1/2 that dose rate (1 ml/kg) and observed.

   b. If no deaths are observed, the remaining rabbits will be injected at the initial 2 ml/kg dose, alternating control rabbit and test rabbit, and observed.

2. If any deaths occur following the injections, a veterinarian/pathologist will perform a post-mortem.

F. Fourteen (14) days after the dosing solution is injected, the rabbits will be killed:

1. Weigh the rabbit (final body weight).

2. Anesthetize the rabbit with the sodium pentobarbital, dosed at 40 mg/kg, into the lateral ear vein using a 25 gauge needle and 3 ml syringe.

3. A heart puncture is then performed using a vacutainer cuff/needle and vacutainer tubes to collect the blood samples for hematology testing (Superchem and CBC with differential, see Section IX, Part D, #1).

4. Overdose the rabbit with sodium pentobarbital until death occurs.

5. Collect and examine grossly the following organs: Brain, Heart, Lungs, Thymus, Spleen, Kidneys (both), Liver and Testes (both) in the animal and upon removal.

6. Weigh each organ and record the weight (the weighing boats have been pre-weighed, their weights recorded and this weight needs to be subtracted from the combined organ and weighing boat weight to obtain organ weight).

7. Section organs (except thymus), if needed, for pathology (see Section IX, Part F, #1) and place the whole organ or representative organ sections in formalin.

8. Place carcass and remaining organs in plastic bag for incineration; clean area and instruments after each rabbit is sacrificed.

9. Prepare blood samples as described in Section IX, Part D, #2.

10. The organs and organ sections will be allowed to fix in the formalin for at least 24 hours before smaller sections are selected and cut to fit the histological cassettes for embedding.

11. Deliver the sectioned tissues in formalin to the Pathology Department, SUNY at Buffalo for histological preparation (See Section IX, Part F, #3).

VII. External Factors

A. Animal Diet

1. ProLab High Fiber Rabbit 5P25

Guaranteed Analysis:

Crude protein not less than	16.0%
Crude fat not less than	2.0%
Crude fiber not less than	19.0%
Crude fiber not more than	24.0%
Calcium not less than	0.8%
Calcium not more than	1.3%
Phosphorus not less than	0.5%
Salt (NaCl) not less than	0.5%
Salt (NaCl) not more than	1.0%

Vitamin A not less than	5000.0 IU/Lb
Ash not more than	6.5%
Added minerals not more than	3.5%

2. The rabbits will be fed on the following schedule which is on the recommendations of HRP, Inc.:

First 12-24 hours -	NO FOOD
Day 1 -	25 grams
Day 2 -	50 grams
Day 3-4 -	75-100 grams
Day 5-7 -	100-125 grams

Note: Restricted feeding will not restrict growth rates because nutritional requirements are met at 125 grams.

3. Water will be available from the automatic watering system that is attached to each cage rack, with a water spigot available to each rabbit. The water is obtained from the City of Buffalo's public water system (tap water). The water spigot will be checked daily to assure that water is available to each cage.

B. Control and Test Articles

The sponsor of this project is responsible for the specifications of the control and test articles, with concern for contaminants that could reasonably be expected to be present and capable of interfering with the purpose of this study.

VIII. Administration of Control/Test Articles

A. Dosage Level

The control and test rabbits will receive one (1) injection that will be administered intravenously at a dose of 2 ml/kg of body weight. If acute toxicity is observed, then reduce the dose to 1 ml/kg for both the control and test articles. A reference for a maximum bolus dose of 2 ml/kg is recommended in Principles and Methods of Toxicology, 2nd Edition, Editor: A. Wallace Hayes, Raven Press, New York, 1989, p. 862. This study dose does not exceed this recommendation.

B. Method

The test and control articles will be administered in an alternating pattern (control rabbit, test rabbit, control rabbit, etc.) with an intravenous injection in the lateral ear vein.

1. Properly restrain the rabbit (hand-held or in a commercial rabbit restrainer).

2. Prepare lateral ear vein by applying 70% alcohol to area.

3. Stimulate blood flow to vein with sharp finger-flicks to area.

4. Insert the 25 gauge needle attached to an appropriately sized syringe (1 ml or 3 ml) into the ear vein.

5. Inject the control and test articles cautiously, but at a reasonable rate (average = 1-2 minutes).

6. Remove needle/syringe from the vein and apply pressure to area with a 2x2 gauze until bleeding stops.

IX. Type/Frequency of Tests

A. Scale Calibration - To be performed on a pre-weighing and post-weighing basis when the rabbits are weighed, when the weighing boats are weighed, and when the rabbit organs are weighed on the day of sacrifice.

B. Body Weight Gain - The rabbits will be weighed Monday through Friday during the one week quarantine period and during the two week study period.

C. Monitoring

1. Physical - The rabbits will be observed daily for any changes in food or water consumption and for tissue reactions at the site of the injection.

2. Toxicological - The rabbits will be observed after the injection for signs of acute toxicity as described in Principles and Methods of Toxicology, 2nd Edition, Editor: A. W. Hayes, 1989, p. 180-181.

D. Clinical

1. On the day of the kill (fourteen days after the dosing injection) the following blood samples will be drawn with a heart puncture, after the rabbit is anesthetized intravenously with sodium pentobarbital, for testing:

   a. 4 ml EDTA vacutainer tube for CBC with differential (ANTECH Diagnostics Test #951); invert tube a minimum of ten (10) times to mix.

   b. 4 ml SST (Serum Separator Tube) vacutainer tube for diagnostic Superchem screen (ANTECH Diagnostics Test #951); invert tube five times to mix the clot activator and blood, allow blood to clot for at least 20 minutes, then centrifuge at full speed for 15 minutes.

2. Sort each rabbit's labeled EDTA tube and labelled Serum Separator Tube with a completed ANTECH Diagnostics Test Requisition form (ANTECH Diagnostics Account Number #31104260-6) into a plastic bag (one per rabbit); place specimen bags into ANTECH Diagnostics Shipping Box (provided); call FED EX at 1-800-463-3339 for pick-up.

3. Samples will be transported by FED EX to ANTECH Diagnostics (Phone: 1-888-397-8378) for testing.

E. Organ Weights - On the day of the kill (fourteen days after the dosing injection) the following body organs will be examined grossly for abnormalities, collected, and their weights recorded:

1.	Thymus	Small weighing boat
2.	Lungs (both)	Medium weighing boat
3.	Heart	Small weighing boat
4.	Spleen	Small weighing boat
5.	Kidneys (both, peel off capsule before weighing)	Medium weighing boat
6.	Liver	Medium weighing boat
7.	Testes (both)	Small weighing boat
8.	Brain	Small weighing boat

Note: The weighing boat size is selected to accommodate the total organ and is pre-weighed

F. Histological Preparation

1. After the specified organs have been weighed, the organs will be placed into a jar containing formalin. Each jar will be pre-labeled with the rabbit's study number and the date. The organs will be prepared for the fixative process by placing them in the formalin in the following manner:

   a. Lungs - with scissors/mid-section slice (from 2 lobes)

   b. Heart - whole organ into fixative

   c. Spleen - whole organ into fixative

   d. Kidneys - with razor blade/butterfly each

   e. Liver - with razor blade/mid-section slice (from 2 lobes)

   f. Testes - whole organs (both) into fixative

   g. Brain - whole organ into fixative (use separate formalin jar)

2. The organs or organ sections will be allowed to fix in the formalin for at least 24 hours; the fixed organ or organ section will then be removed from the formalin and sectioned into properly sized pieces to fit into the histological cassette used during the embedding process.

3. The formalin jars containing the sectioned organs and a Histological Preparation Request Form, will be delivered to the Pathology Department, School of Medicine, SUNY at Buffalo, for processing:

Request Form includes:

a. Dehydration and Embedding -R

b. Sectioning - 5 um

c. Stain - H&E

4. Histology slides will be read by the pathologist, Peter A. Nickerson, A.B., M.A., Ph.D. - Professor and Director of the Pathology Graduate Program, 212 Cary Hall, SUNY at Buffalo. The complete pathology procedure is attached to the end of this protocol.

X. Records

A. "Study 28" Data Notebook will be kept in Farber Hall - Room 118G, SUNY at Buffalo:

1. Inventory of control and test articles received from the sponsor and the animals on which it was administered; paper work for returned control and test articles to the sponsor.

2. Information on rabbits: Shipment information, initial weights, sex, physical condition.

3. Scale calibration: Scales used for rabbit weights, organ weights, and weighing boat weights.

4. Rabbit body weights, with a group mean weight ± Standard Deviation.

5. Daily physical observations of rabbits.

6. Injection day data: Date, Rabbit weights, Dose volume, Time, Vial #of article injected, Physical observations (signs of acute toxicity), Post-mortem report of any deaths.

7. Weight of organs at sacrifice: Brain, Thymus, Lungs, Heart, Spleen, Kidneys, Liver, Testes.

8. ANTECH Diagnostics Test Request Form (copy) for the clinical diagnostic tests to be performed on each rabbit.

9. Histology Request Forms for the tissue specimens submitted for pathology.

10. Hematology test results (Superchem and CBC with differential) on each rabbit, as performed by ANTECH Diagnostics.

11. Compilation chart of hematology test results.

12. Compilation chart of organ weights with organ/body weight ratios for each rabbit.

13. Pathology report of histology slides, as prepared by Dr. Peter A. Nickerson.

14. Statistical findings.

B. Histology tissue blocks.

C. Histology slides.

XI. Approval of Protocol

A. By Sponsor: R.E. Counsell 8/21/98
Dr. Raymond Counsell Date

B. By Study Director: Paul Kost 6/2/98
Dr. Paul J. Kostyniak Date

XII. Statistical Methods

Differences in body weights and biochemical parameters will be compared between groups using the t-test

XIII. Revisions

Any revisions made to this protocol will be attached in the appendices.

XIV. Materials and Equipment

A. Scales

1. Ohaus PB-30(Serial #2295) - Measures to 0.005 kilograms (kg), from the Laboratory Animal Facilities, SUNY at Buffalo.

2. Sartorius 1212 MP (Serial #2907085) - Measures to 0.001 grams (g), from the Toxicology Research Center, SUNY at Buffalo.

B. Centrifuge - Dynac Benchtop Centrifuge #0101 (Serial #22424), from the Toxicology Research Center, SUNY at Buffalo.

C. Syringes - BD syringes 1 ml and 3 ml Single dose, Sterile, with Luer Lock tip, purchased from the Laboratory Animal Facilities, SUNY at Buffalo.

D. Needles

1. Monoject 25 gauge hypodermic needles X 5/8" long, purchased from the Laboratory Animal Facilities, SUNY at Buffalo.

2. Monoject 20 gauge hypodermic needles x 1" long, purchased from the Laboratory Animal Facilities, SUNY at Buffalo.

Note: Monoject needles are sharper than BD needles

3. Precision Glide Vacutainer Brand Blood Collection 21 gauge x 1", obtained from Lab Corp of America.

E. Instruments - Large dissection scissors, Small dissection scissors, Scalpel handle #4, Scalpel blades #21, Forceps, Rongeur, Spoon, Single-edged razor blades.

F. Drugs - Sodium Pentobarbital @ 65 mg/ml, manufactured by Veterinary Laboratories, Inc. for the Butler Company, Lot #980410 970789, Expiration Date - 2/00 & 6/00. This will be purchased from the Laboratory Animal Facilities, SUNY at Buffalo, on an ml's as needed basis.

G. ANTECH Diagnostics

1. 4 ml EDTA vacutainer tube (Lavender top), Becton Dickinson #366405, Lot #8C232, Expiration Date: MAR00.

2. 4 ml Serum Separator Tube (SST Vacutainer - Red/Gray top), Becton Dickinson #366514, Lot #8D907, Expiration Date: MAR99.

3. Plastic bags for transporting specimens (a bag for each animal's blood tubes).

4. ANTECH Diagnostics Hematology Request Forms with pre-printed Toxicology Research Center Account #31104260-6 and address.

5. FED EX Shipping boxes

H. Miscellaneous

1. Lab table soaker paper for kill (individual pieces per rabbit), rolls purchased from Biochemistry Stockroom, Farber Hall - Room 10, SUNY at Buffalo.

2. Glass cutting board for sectioning organs for pathology, Toxicology Research Center, SUNY at Buffalo.

3. Polystyrene weigh boats

   a. Small: 37x10 mm, Laboratory Products Sales (Catalog # D205-1), purchased from Biochemistry Stockroom, Farber Hall - Room 10, SUNY at Buffalo.

   b. Medium: 78x19 mm, Laboratory Products Sales (Catalog # D205-2), purchased from Biochemistry Stockroom, Farber Hall - Room 10, SUNY at Buffalo.

4. Vacutainer cuff for drawing blood into vacutainer tubes, from Toxicology Research Center, SUNY at Buffalo.

5. Formalin jars - Nalgene, 250 ml (8 oz.) with polypropylene cap, from VWR Scientific Products, Catalog #16129-378.

6. Formalin - "Z-Fix" prepared by and obtained from Pathology Department, Farber Hall - Room 202C, SUNY at Buffalo (Concentrate from Anatech Ltd, 1020 Harts Lake Road, Battle Creek, Michigan 49015, Phone: 1-800-Anatech).

7. Disposable latex exam gloves, purchased from Biochemistry Stockroom, Farber Hall - Room 10, SUNY at Buffalo.

PATHOLOGY PROCEDURES

The contents of the jar containing tissue from one animal are poured into a sieve. The formalin solution is drained off and collected into a separate container for proper disposal. Technicians in the histology laboratory prepare two processing plastic cassettes with the exact number that matches the number on the jar and the number on the list. The liver, lung, heart, spleen, kidney and testes are sliced with a safety razor blade to a thickness of 2 mm and placed into the cassette. The cassette is then dropped into a running water bath. The brain is sliced transversely to include the basal ganglia in the cerebrum and the cerebellum. The brain tissues are processed in a similar manner by being placed in a cassette and dropped into the water bath.

The tissues are processed by standard procedures for preparation of histological sections: dehydration through several concentrations of alcohols, xylene and paraffin embedding with a 5 micron section fixed on a microscope slide. The slide is then stained with hematoxylin and eosin and cover slipped. The identification number is consistently marked on each slide.

Each slide is microscopically examined:

1. The number of the slide is recorded, the entire section on each slide is surveyed at low power (40 X), for orientation of the section, histological components and any abnormalities visible at this magnification

2. The entire section is viewed with medium power (100 X), and any abnormalities are noted.

3. With high dry power (400 X) the individual histologic components of the section are carefully examined and any abnormality differing from the normal histological appearance is noted and recorded.

After reading all the slides and recording histopathological changes, general comments and comparisons are made. A report on the findings is prepared, signed and presented to the Toxicology Research Center.

Peter A. Nickerson

Peter A. Nickerson, Ph.D.
Professor of Pathology

Appendix 5: Letter from Food and Drug Administration and I.N.D. Number

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20857

IND 62,703

Milton Gross, M.D.
Professor, Radiology and Internal Medicine
B1G 505C, University Hospital
1500 E. Medical Center Dr.
Ann Arbor, MI 48109-0028

Dear Dr. Milton:

Please refer to your Investigational New Drug Application (IND) submitted under section 505(i) of the Federal Food, Drug, and Cosmetic Act for NM-404.

We have completed our 30-day safety review of your application and, as discussed with your representative, Dr. Marc Longino, in the teleconferences on June 25 and 26, 2001, have concluded that you may proceed with your proposed clinical investigation.

If we have any comments to relay to you, we will send them to you in a separate letter or fax.

As sponsor of this IND, you are responsible for compliance with the Federal Food, Drug, and Cosmetic Act and the implementing regulations (Title 21 of the Code of Federal Regulations). Those responsibilities include (1) reporting any unexpected fatal or life-threatening adverse experience associated with use of the drug by telephone or fax no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)]; (2) reporting any adverse experience associated with use of the drug that is both serious and unexpected in writing no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)]; and (3) submitting annual progress reports (21 CFR 312.33).

Please forward all future communications concerning this IND in triplicate along with Form FDA 1571, identified by the above IND number, to the following address:

U.S. Postal Service/Courier/Overnight Mail:
Food and Drug Administration
Center for Drug Evaluation and Research
Division of Medical Imaging and Radiopharmaceutical Drug Products
Attention: Division Document Room, 18B-06
5600 Fishers Lane, HFD-160
Rockville, Maryland 20857

If you have any questions, call Thuy M. Nguyen, M.P.H., Regulatory Health Project Manager, at (301) 827-7510.

Sincerely,

{See appended electronic signature page}

Patricia Y. Love, M.D., M.B.A.
Director
Division of Medical Imaging and
Radiopharmaceutical Drug Products
Office of Drug Evaluation III
Center for Drug Evaluation and Research

Appendix 6: Presentations and Publications

Radiopharmaceutical Chemistry Track
Dosimetry: Clinical Dosimetry

2:15 PM-3:45 PM **Session 23** **Room: 403 B**

Moderator: Barry W. Wessels, PhD
Co-Moderator: John L. Humm, PhD

No. 155

PREDICTED DOSIMETRY FOR I-131-NM-404, A PHOSPHOLIPID ETHER AGENT FOR TUMOR IMAGING AND POSSIBLE THERAPY. K. R. Zasadny*, M. A. Longino, S. J. Fisher, R. E. Counsell, R. L. Wahl, The University of Michigan Medical Center, Ann Arbor, MI. (500384)

Objectives: Phospholipid ether agents have the potential to image and possibly deliver therapeutic radiation to a wide variety of human tumors due to their differentially slower metabolism in tumors relative to normal tissues. Previous phospholipid ether agents have successfully targeted a variety of human neoplasms including colon, lung and ovarian cancer. Iodine-labeled NM-404 has successfully targeted tumors in the rat including the Walker256 tumor line. This study focuses on predicted normal organ dosimetry for I-131-labeled NM-404 for humans based on biodistribution studies in the rat. Methods: Tissue distribution studies were carried out after I-125-labeled NM-404 injection in male Sprague-Dawley rats at six time points (3 animals per time point): 1 hr, 6 hr, 24 hr, 72 hr, 7 d and 10 d post injection. Kg*%ID/g uptake in tissues were calculated. Time-activity curves were fit by non-linear least-squares regression using a biexponential model. Extrapolation to human was accomplished by scaling by the total body and organ masses of the MIRD reference adult phantom. Fit time-activity curves were corrected for I-131 decay and integrated to determine dosimetric residence times for the following source organs: adrenals, heart, kidneys, liver, lungs, muscle, marrow, spleen, testes and thyroid (unblocked). The MIRDOSE 3.1 program was used to produce dose estimates. Results: The NM-404 pharmacokinetics show a rapid clearance from the blood followed by a long-lived component. Normal tissues generally show rapid uptake followed by slow clearance. Highest normal organ dose estimates (mGy/MBq) for I-131-labeled NM-404 for the reference adult were seen in thyroid (unblocked) (0.82), followed by adrenals (0.61), lungs (0.56), kidneys (0.50), spleen (0.41), testes (0.39) and liver (0.34). The dose-limiting organ is the testes, with a 3 cGy dose resulting from a 78 MBq administration. Conclusion: Predicted I-131-labeled NM-404 dosimetry results indicate clinically-useful activities for imaging may safely be injected in humans with thyroid blocking. Phase I studies in humans are planned using a 74 MBq (2 mCi) dose.

No. 156

OPTIMIZING COMBINATION THERAPY WITH RADIOLABELED ANTIBODIES AND EXTERNAL BEAM. J. L. Humm*, S. Ruan, S. M. Larson, J. A. O'Donoghue, Memorial Sloan-Kettering Cancer Center, New York, NY. (100338)

Objective: To determine the optimum sequence for combined modality therapy with radiolabeled antibodies and fractionated external beam. **Methods:** The uptake and distribution of I-131 labeled tumor specific A33 monoclonal antibody was determined in SW1222 human colon carcinoma xenografts in nude mice for four study groups (4 animals per group): (1) radiolabeled antibody alone, i.e. pre-radiation therapy controls, (2) antibody administered (day 0) immediately prior to the first of five 2 Gy daily fractions of 320 kVp X-rays, (3) antibody administered after the 5th radiation fraction (day 5), (4) antibody administered five days post irradiation (on day 10). Tumors were excised 5 days post antibody administration. The %injected dose per gram was calculated. Tumors were frozen and sectioned for histology and phosphor imaging autoradiography. The percentage of antigen expressing cells was measured by immunohistochemistry. **Results:** The average tumor uptake relative to control group 1 were 1.47 (group 2), 0.78 (group 3) and 0.21 (group 4) respectively. This illustrates that tumor uptake is increased by almost 50% when the antibody is present in blood at the start of irradiation. 5 days into a fractionated irradiation protocol, antibody uptake was reduced, falling more significantly on day 10. Autoradiographs demonstrated decreased uptake uniformity for groups 3 and 4. Immunohistochemistry showed a reduction in A33 antigen positive cells from 85, 64, 50 to 41% for groups 1-4 respectively. **Conclusions:** Radioimmunotherapy should be administered just prior to the initiation of a course of external beam for maximum tumor uptake and radiolabeled antibody dose. Radiation therapy appears to cause a transient increase in capillary leakage to macromolecules, followed by a reduction at later times possibly the result of capillary damage and occlusion.

No. 157

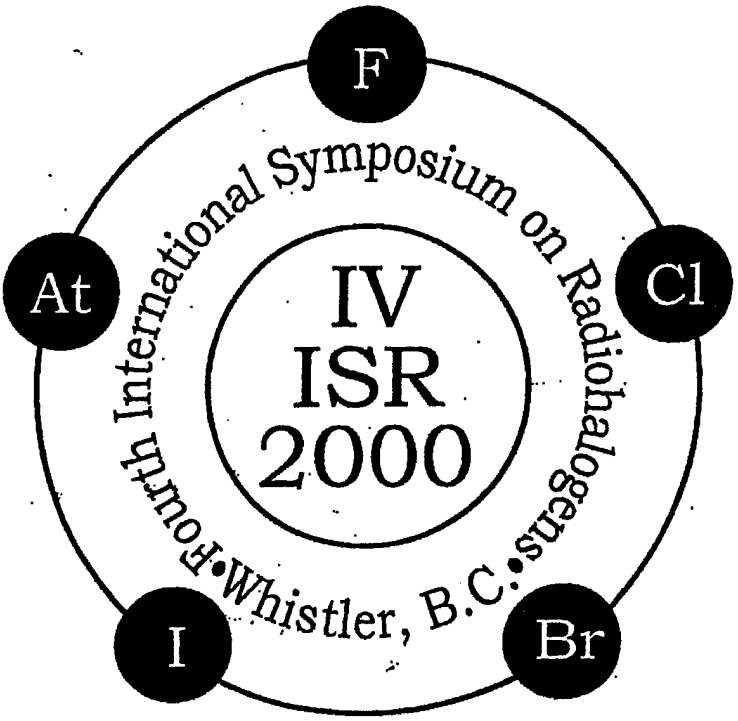
NEW ADDITIONAL MIRD MODEL BASED ADULT PHANTOMS OF DIFFERENT SIZE FOR INTERNAL DOSIMETRY IMPROVEMENT. I. Clairand*, M. Ricard, M. Durigon, M. Di Paola, B. Aubert, Institut Gustave-Roussy, Villejuif, France; Institut Gustave-Roussy and U494 INSERM, Villejuif, France; Hopital Raymond-PoincarZ, Garches, France. (500325)

Objectives: In internal dosimetry, patient morphology is represented by a limited number of models. In the MIRD schema, the adult male phantom is an individual measuring 1.74 m and weighing 70 kg, the adult female is represented by 1.64 m and 58 kg. In order to work with more realistic models, we defined additional MIRD based mathematical anthropomorphic phantoms which represent the physical differences encountered in the adult population. The influence of these morphologic variations on the S-factors was studied. Methods: The analysis of anthropometric data gathered from a legal medicine department (355 men and 329 women of Caucasian type) showed that the mass of most organs is statistically correlated with the height of the body. This led us to develop 3 mathematical male phantoms of 1.60 m, 1.70 m and 1.80 m and 3 female phantoms of 1.50 m, 1.60 m and 1.70 m. These phantoms were built using combinatorial geometry. The S-factors for all the usual target organs were then calculated using a home made Usercode DOSE3D based on the EGS4 Monte Carlo code, when I-131 is uniformly distributed in the stomach and the urinary bladder. Results: An increase in the phantom height by 10 cm leads to a mean S-factor reduction by 20 % when the stomach is the source organ and by 29 % in the case of the urinary bladder. When the phantom height increase is 20 cm, the values are 35 % and 48 %. In some cases, especially when the target organ is far away from the source organ, the differences are 4 fold or more. Conclusion: This work showed the influence of the morphology on the S-factors. The development of new mathematical adult phantoms should contribute to improve dosimetric estimations by taking into account more realistic geometric parameters.

No. 158

ERROR ANALYSIS OF GAMMA CAMERA BASED DOSIMETRY IN RADIOIMMUNOTHERAPY. K. A. Hamacher*, G. Sgouros, Memorial Sloan-Kettering Cancer Center, New York, NY. (100032)

Objectives: The aim of the work presented here was to implement a detailed method to evaluate the error associated with the calculation of the absorbed dose to normal organs in patients undergoing radioimmunotherapy. Methods: The overall uncertainty in absorbed dose is assumed to include errors in (1) estimation of organ activity at multiple time-points from radionuclide imaging and (2) estimation of organ volume. Organ activity quantification is comprised of the following measurements, each of which will have its own uncertainty: attenuation correction, scatter correction, camera calibration, selection of an appropriate background region-of-interest, and selection of a region-of-interest for the organ. Several of these measurements are comprised of a number of independent measurements which themselves are subject to uncertainty. The uncertainty in organ volume quantification will be highly dependent upon the technique used to estimate organ volume with CT or MRI-based measurements being the most accurate and estimation based upon nuclear medicine imaging being less accurate. Error values were assigned to each of the measurements identified above and then propagated to obtain the uncertainty in calculated absorbed dose. Uncertainties were calculated assuming dosimetry was based upon imaging In^{-111}, I^{-131}, or Bi^{-213}. Uncertainty values were determined for volume estimates based upon CT/MRI, SPECT and also estimates based upon organ projections obtained from planar imaging. **Results and Conclusion:** A formalism has been established which provides the uncertainty associated with conventional absorbed dose calculations. This analysis makes it possible to quantitatively identify those elements that contribute the largest uncertainty to absorbed dose estimates, thereby pointing to areas where improvement would be most beneficial.



Fourth International Symposium on Radiohalogens

Whistler, B.C., Canada

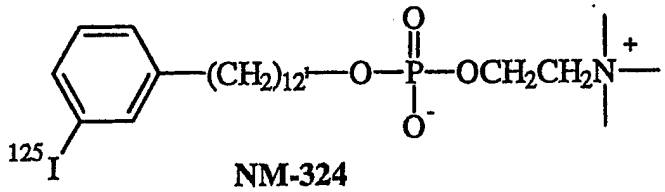
September, 9 - 13

2000

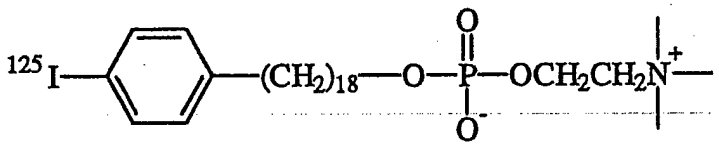
Radioiodinated Phospholipid Ethers and Analogs as Tumor Imaging Agents

R.E. Counsell, M.A. Longino, M.E. Van Dort, S.J. Fisher, A.N. Pinchuk, R.W.S. Skinner, K.R. Zasadny and R.L. Wahl.
Departments of Pharmacology, Radiology and Internal Medicine.
The University of Michigan Medical School, Ann Arbor, Michigan, 48109 U.S.A.

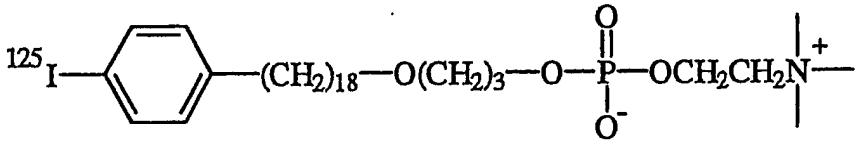
Based upon reports that human tumor tissue contains significantly higher levels of phospholipid ether (PLE) than adjacent normal tissue, our laboratory designed and synthesized a number of radioiodinated PLE analogs as potential tumor imaging agents. Several of these agents showed a striking ability to be taken up and retained by a variety of animal tumors and human tumor xenografts. In an effort to establish the relevance of our animal models to the human situation, one candidate (NM-324) was selected for further preclinical evaluation and subsequently studied in cancer patients. Such studies revealed that NM-324 was capable of imaging tumors in patients, but the high first pass clearance by the liver severely compromised its clinical utility as a diagnostic radioiopharmaceutical. Conversely, this study demonstrated that our animal models were appropriate for the identification of clinical candidates. Therefore, the design of second-generation candidates was focused on those that would possess a longer plasma half-life and/or more rapid metabolic clearance by the liver and other non-target tissues. Two animal models were employed for these studies, namely: SCID mice bearing 1) human lung adenocarcinomas (A549) and 2) human prostate cancer (PC-3). Based upon biodistribution and whole body imaging, two candidates (NM-404 and NM-412) were observed to be superior to NM-324. Moreover, toxicological analysis has shown both NM-404 and NM-412 to have no physiologic or pathologic effects in rats or rabbits at a dose significantly greater than 200 times the anticipated human dose. Phase I trials with both these agents in cancer patents are planned.



NM-324



NM-404



NM-412

TRIUMF

Proceedings of the 3rd International Conference on Isotopes

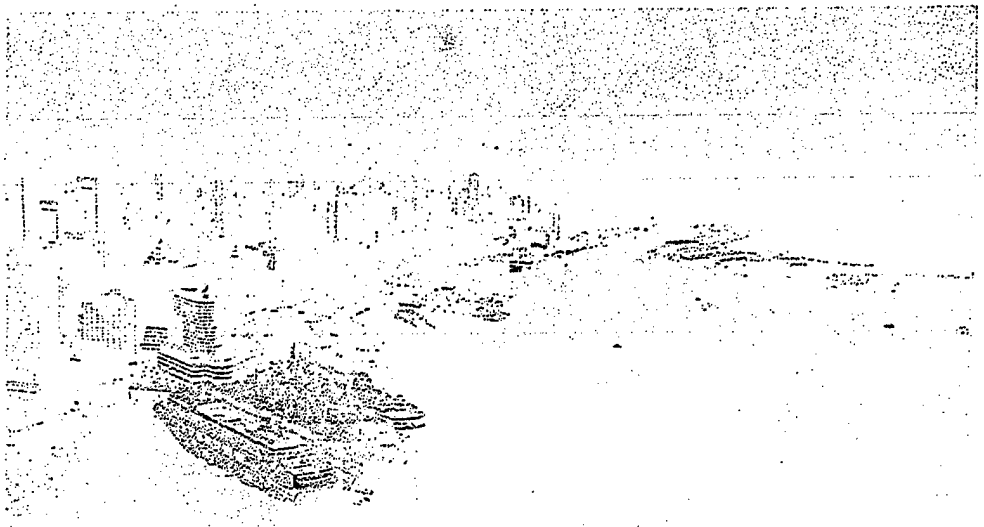
Isotope Production and Applications in the 21st Century

Vancouver, Canada

6 – 10 September 1999

Editor

Nigel R. Stevenson



World Scientific

SYNTHESIS AND EVALUATION OF A RADIOIODINATED PHOSPHOLIPID ETHER ANALOG (NM-404) FOR DIAGNOSTIC IMAGING OF PROSTATE CANCER

R.E. COUNSELL

Department of Pharmacology, The University of Michigan Medical School, Ann Arbor, MI, 48109 U.S.A.

E-mail: counsell@umich.edu

M.A. LONGINO, A.N. PINCHUK, R.W.S. SKINNER, S.J. FISHER, M.E. VAN DORT, K.J. PIENTA AND R.L. WAHL

Departments of Internal Medicine, Pharmacology, Radiology and Surgery, The University of Michigan Medical School, Ann Arbor MI, 48109 U.S.A.

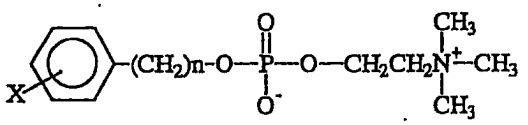
Imaging procedures play a major role in the current management of patients with prostate cancer. Despite advances in many of these procedures, improvements are still needed, especially in the area of Nuclear Medicine. The radioiodinated phospholipid ether analog (PLE) described here represents a new class of radiopharmaceutical, which has provided excellent images of prostate tumors in animal models and is now undergoing preclinical human pharmacokinetic evaluation.

Design and synthesis of radioiodinated PLE was based on the fact that various animal and human tumors contain higher concentrations of ether lipids than surrounding normal tissues. A number of radioiodinated PLE were synthesized and evaluated by γ-camera imaging using rat tumor models as well as nude and SCID mice bearing human tumor xenografts. Of the several agents that displayed promising results, one candidate (NM-324) was selected for further preclinical evaluation and subsequently studied in cancer patients in an effort to ascertain its ability to be retained in human tumors. These studies revealed that NM-324 was capable of imaging tumors in patients, but the high first pass clearance by the liver severely compromised its clinical utility as a diagnostic radiopharmaceutical. Conversely, this study demonstrated that our animal models were appropriate for the identification of clinical candidates.

In an effort to obtain a more suitable clinical candidate, the present study undertook the synthesis and evaluation of additional radioiodinated PLE with a focus on those displaying good tumor avidity and a prolonged plasma half-life relative to the prototype. Biodistribution analysis and γ-camera imaging of Copenhagen rats bearing Dunning R3327 prostate tumors and SCID mice bearing human prostate cancer (PC-3) revealed NM-404 to display a longer plasma half-life, better tumor/liver and tumor/kidney ratios, and significantly superior imaging properties than the initial prototype, NM-324. (Supported by the U. S. Department of Defense grant DAMD17-98-1-8528 and the SPORE in Prostate Cancer grant P50 CA 65968)

We previously described the remarkable capacity of certain radioiodinated phospholipid ether (PLE) analogs to be selectively retained by a variety of rodent and human tumor cell lines [1]. Moreover, this property made it possible to obtain images of these tumors in rabbits, rats and mice using γ-camera scintigraphy. Based

on these and other preliminary results, one of these radioiodinated analogs, 12-(*m*-iodophenyl)dodecyl phosphocholine (NM-324, Figure 1), was approved for pharmacokinetic evaluation in human cancer patients in order to determine whether the results in animals could be confirmed in humans. Although high first pass clearance by the liver compromised the imaging capabilities of NM-324, imaging of the tumors was successful in several patients, and thereby confirmed the potential of radioiodinated PLE analogs for tumor imaging in patients [2].

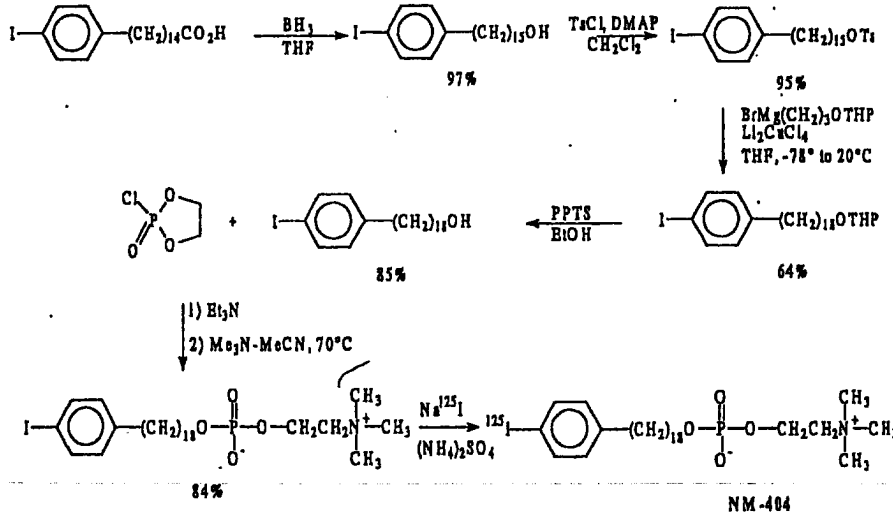


	X	n
NM-324	*m*-^{125}I	12
NM-404	*p*-^{125}I	18

Figure 1. Structures for NM-324 & 404.

In an effort to obtain a more suitable clinical candidate, the present study undertook the synthesis and evaluation of analogs of NM-324 with the aim of improving the tumor retention *vis a vis* the liver and kidneys. Placing the radioiodine in the *para* position and increasing the aliphatic chain length led to NM-404 (Figure 1) which not only increased lipophilicity but also led to the desired properties.

Scheme 1. Synthesis of NM 404



Phosphocholination was performed according to Chandrakumar and Hajdu [3], and radioiodination followed the procedure of Mangner, et al. [4].

Biodistribution analysis (Table 1) and γ-camera imaging was performed in Copenhagen rats bearing Dunning R3327 prostate tumors and in SCID mice bearing human prostate cancer (PC-3). Comparison of NM-324 and 404 over several days revealed that tumor visualization was possible in both instances, but radioactivity was only seen to clear from abdominal organs following administration of NM-404.

Based on these results, NM-404 was selected for further preclinical analysis. The Toxicology Research Center at the University Buffalo found an isotonic solution of stable NM-404 to have no physiologic or pathologic effects in rats or rabbits at a dose 200 times the anticipated human dose. Moreover, the above tissue distribution studies along with those in normal Sprague-Dawley rats predicted that ^{131}I labeled NM-404 could be safely injected in humans with thyroid blocking at a dose of 2 mCi.[5]. Phase I studies in humans are planned.

Table 1. Biodistribution of ^{125}I-NM-404 in male SCID mice bearing PC-3 human prostate cancer xenografts, expressed as Dose/gm ± SEM and Target/Non-target Ratio, (n=4).

	1 DAY	3 DAY	5 DAY	8 DAY
Tissue	% Dose/gm (Tumor/Tissue)	% Dose/gm (Tumor/Tissue)	% Dose/gm (Tumor/Tissue)	% Dose/gm (Tumor/Tissue)
Blood	5.74±0.20 (1.59)	3.10±0.13 (4.24)	3.08±0.09 (5.87)	2.17±0.07 (6.91)
Kidney	4.22±0.14 (2.17)	2.14±0.11 (6.13)	2.28±0.09 (7.92)	1.46±0.04 (10.26)
Liver	3.69±0.21 (2.48)	1.93±0.10 (6.81)	1.63±0.06 (11.07)	1.02±0.06 (14.69)
Lung	5.36±0.33 (1.71)	2.60±0.20 (5.06)	2.27±0.09 (7.97)	1.54±0.06 (9.70)
Muscle	0.79±0.03 (11.50)	0.57±0.04 (22.98)	0.49±0.03 (36.95)	0.40±0.03 (37.33)
Prostate	2.60±0.15 (3.51)	1.40±0.27 (9.40)	1.96±0.25 (9.20)	1.41±0.06 (10.64)
Tumor	9.14±0.69 (1.00)	13.14±0.40 (1.00)	18.06±0.80 (1.00)	14.96±0.63 (1.00)

References

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5. Zasadny K. R., Longino M. A., Fisher S. J., Counsell R. E. and Wahl R. L., Predicted dosimetry for I-131-NM-404, a phospholipid ether agent for tumor imaging and possible therapy, *J. Nucl. Med.* 40 (1999) p. 39P.